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U.S. National Cancer Institute.
" Report of program activities.

Serial No. NCI-433
1. Laboratory of Biology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the viral etiology of neoplasms

Principal Investigator: Sarah E. Stewart

Other Investigators: B. E. Eddy, BS-LVP,NIH

Cooperating Units: Sloan-Kettering Institute, N. Y.

Man Years (calendar year 1959):
Total: 5
Professional: 1
Other: 4

Project Description:

A. Work on Polyoma virus:

1. Development of resistance to S E polyoma virus.

In the early work with leukemia cell-free extracts it was observed that of several litters of newborn mice inoculated with certain preparations, all mice in a given litter might develop tumors while the rest of the litters remained negative. Such results have been interpreted as a litter effect by Law et al (J. Nat. Cancer Inst. 16: 495, 1955). We have now shown that this all-or-none response was probably not due to a peculiarity of the litter of mice inoculated but was due to the mother of the litter. In many laboratories where the tumor-inducing agent was not considered as an infectious virus, the female mice that had nursed an inoculated litter were reused for breeding. In our experience mice that had nursed infected litters were never reused (J. Nat. Cancer Inst. 15: 1391, 1955) and with tissue culture propagated polyoma virus 80-100 percent of hybrid (C3Hf/Hen X AKR)F_1 mice injected developed multiple types of neoplasms (J. Nat. Cancer Inst. 20: 1223, 1958).

In more recent studies (C3Hf X AKR)F_1 hybrid mice were found resistant to the oncogenic effects of polyoma virus after it had been carried for 3 to 13 serial passages in tissue cultures made from Swiss mouse embryos, whereas Swiss mice retained full susceptibility to the virus. It thus appeared that while the virus had become adapted to the Swiss mouse by passage in Swiss mouse embryo cultures, it had lost the ability to infect the hybrid mice. Experiments were performed to explore this possibility. It was found that the resistance to the oncogenic effects of polyoma virus after it had been carried for 3 to 13 serial passages in tissue cultures made from Swiss mouse embryos, whereas Swiss mice retained full susceptibility to the virus. It thus appeared that while the virus had become adapted to the Swiss mouse by passage in Swiss mouse embryo cultures, it had lost the ability to infect the hybrid mice. Experiments were performed to explore this possibility. It was found that the resistance of the hybrid mice was due to maternal antibodies transmitted to them in the milk of their C3Hf/Hen mothers. Although the strain C3H/Hen had not previously nursed an inoculated litter, they were housed in an environment contaminated with polyoma virus while the Swiss mice were not. The milk of mice which had been inoculated with polyoma virus had as high hemagglutination inhibition titers as were found in their sera.

In a colony of mice with latent polyoma virus infection transfer of maternal antibodies to the offspring by way of milk may account for any irregularity of results following inoculation of this virus. Maternal antibodies may also explain the lack of spontaneous parotid gland tumors in an infected colony, as it is common for newborn mice to nurse almost immediately after birth. Maternal antibodies transferred to the offspring may also explain the absence of these neoplasms in the offspring of tumor bearing females.

2. Tissue culture plaques of S E polyoma virus.

The cytopathogenic changes produced by S E polyoma virus in mouse embryo cultures make it possible to apply the tissue culture plaque method for determining if only one virus produces the spectrum of neoplasms observed in inoculated mice. Dr.B. Eddy has shown that in hamsters plaqued virus produced the same types of neoplasms as that not plaqued. Since in hamsters the virus produces only sarcomas and vascular lesions the results are not as conclusive as they would be in mice.

Polyoma virus used in this study was subline #6069 derived from a Swiss mouse that had been injected shortly after birth with virus subline #3b69 which had been passed through a 220 mu. gradacol filter. During a 2 month period the mouse developed primary neoplasms, many of which were multiple. These consisted of multiple mammary adenocarcinomas, hair follicle epidermoid carcinomas, epidermoid carcinoma of the gingiva, pleomorphic tumors of the parotid, exorbital lacrunal and tracheal submucosal glands, thyroid carcinoma epithelial thymoma, mesotheliomas of the pleura, renal sarcoma, renal tubular lesions, and osteogenic sarcomas of the skull and spine. The mouse was killed when 66 days old and a culture was made in mouse embryo cells from a minco of one of the parotid tumors and one of the mammary adenocarcinomas. The supernatant fluid from the 8th passage in mouse embryo cultures was used in the plaque studies. The technique for plaquing has been described by Dr. Eddy (Proc. III Canadian Cancer Conf. 1959).

Fluids from cultures made from 6 different plaques inoculated into newborn Swiss mice induced tumors in 86 percent. Little variation was noted in the types of tumors observed in mice inoculated with the plaqued and non plaqued virus. Mice that received virus from 3 of the plaques appeared to have a greater variety of tumors than those that received the others; however, the difference between these and the other 3 plaques was probably quantitative rather than qualitative as the majority of the tumors which have been observed appeared in some of the mice.

3. The histogenesis of epithelial thymomas induced in mice inoculated with polyoma virus.

This tumor has been observed in about 30 percent of inoculated mice. It occurs as a circumscribed thymic mass which in small tumors is seen to arise in the medulla. The lesions vary in size from microscopic foci to masses which almost completely displace the lungs. Microscopic examination of serial sections made from early tumors indicate that their origin may be in Hassall's corpuscles. An increase and a hyperplasia of these cells was observed in some early tumors.

4. Chemical nature of S E polyoma virus.

In collaboration with chemists from the Sloan-Kettering Institute and Dr. B. Eddy, NIH, the following results were obtained on the chemistry of polyoma virus:

An infectious nucleic acid resistant to the action of ribonuclease (RNase) but which was inactivated by deoxyribonuclease (DNase) was isolated from mouse embryo cultures infected with polyoma virus. Two methods of preparing nucleic acids from the tissue cultures were employed: that of Gierer and Schramm (Nature 177: 1297, 1956) and the one described by Kirby (Biochem. J. 66: 495, 1957). Both gave the same results. Control nucleic acids were prepared from non infected cultures. Mouse embryo cultures inoculated with the nucleic acid preparations from the infected cultures yielded intact polyoma virus with the RNase treated preparations and not with the nucleic acid treated with DNase. This was demonstrated by cytopathogenic changes in tissue cultures, by hemagglutination of guinea pig erythrocytes and by tumor induction in hamsters and mice. Neither enzyme was found effective on the intact virus.

B. Work with human neoplasms.

1. Cellular proliferation in tissue culture induced with a substance derived from cell-free concentrates from human neoplastic material.

Focal areas of proliferating cells or pocks were produced when cell-free concentrates derived from 2 different human neoplasms and from the urine of 3 children with neoplastic disease were inoculated into monolayered cultures prepared from primary trypsinized cells from human amnion and from human embryo minces. The cells were maintained in Earle's medium 199 (NCTC) containing 5 percent horse serum and antibiotics. The increase in cell multiplication caused them to pile up forming discrete foci sometimes measuring 0.5 cm. in diameter, which were scattered throughout the monolayer of normal cells. The first evidence of cell transformation in both types of cultures was a loss of migration of the cells resulting in a whirl; this is shown in the first figure. The continued growth of these foci in amnion cells is illustrated in figures 2-4.

Cytopathogenic changes were usually observed in the first few tissue culture passages. They were also seen in control cultures which received similar concentrates prepared from human amniotic tissue but were found only in cultures with the original inoculum and after the first passage.

The tumor-cell-free concentrates[3] were prepared by a

3 Whole cells were not observed on examination of the pellets after Spinco centrifugation.

modification of the method described by Melnick (J. Nat. Cancer Inst. 16: 977, 1956), for purification of the Rous sarcoma virus. The urine concentrates were prepared as follows: A morning urine specimen with the pH adjusted to 6.6 was centrifuged in an International refrigerated centrifuge at 3000 rpm (1400 x g) for 30 minutes at 0° C. to remove all cells and gross precipitates. The cleared urine was then centrifuged in a Spinco centrifuge at 40,000 rpm (101,000 Xg) for one hour and the pellets which were usually clear and gelatinous were suspended in a volume of 0.05 M sodium citrate, in which the pH had been adjusted to 6.6 with citric acid, so as to give a 20-fold concentration of the material. The suspended pellets were then homogenized in a Potter-Elvehjem homogenizer and the homogenate was centrifuged at 3,000 rpm (1400 S g) for 30 minutes at 0° C. The supernatant fluid which was generally clear or had only a slight opalescence was inoculated in 1-2 ml. amounts into 2 oz. prescription bottles with the monolayered amnion or human embryo cells and were incubated at 37° C. Tumor concentrates were inoculated in the same manner. Serial passages to other monolayered cultures were made by transfer of 1-2 ml. of culture fluid. The fluids were changed at weekly intervals.

Proliferative changes were produced by five specimens: (1) A surgical specimen from a papillomatous growth of the tongue in a 73 year old male; the patient also had multiple epidermoid carcinomas of the buccal mucosae and a rectal polyp. (2) An autopsy specimen from an embryonal tumor of the liver in a 22 month old child. (3) Urine from a 6 year old boy with acute lymphocytic leukemia. (4) Urine from a 6 year old girl with a renal neuroblastoma. (5) Urine from an 8 year old girl with Hodgkins disease.

The cellular proliferative changes produced by the papilloma extract were observed after the second passage in amnion cell cultures and on the initial culture of the human embryo after 2 weeks' incubation. The cell transformation produced by the liver tumor concentrate was not observed until after the 2nd passage and was not reproducible on all transfers until after it had been carried for 4 weeks in the human embryo cultures.

The cultures inoculated with the urine concentrates were slow in showing a cell transformation and the initial changes were observed only on the human embryo cultures. The original cultures were retrypsinized 3 to 4 times each during a 4-5 month period. Focal areas with abnormal migration of the cells were observed in the inoculated cultures in 2 to 3 months. Transfer of the culture fluid to amnion cultures produced the same focal lesions as observed with the tumor concentrates.

Although serial passage in tissue culture of the proliferative effect is readily accomplished by use of the culture fluids it appears that cellular particles or cells are necessary to produce the effect (even though initially the material was cell-free). Activity is lost by filtration and by centrifugation of the tissue culture fluid. Other instances of similar behavior are known to occur. It is known that in order to transfer varicella virus in tissue culture it is necessary to carry over cell fragments. Also influenza virus has been shown to exist in an incomplete form (non transferable) when grown in certain cells. At present we do not know the nature of the growth accelerating agent but studies are in progress which indicate that the cells transformed by the papilloma agent have a specific antigen which is demonstrable by use of the fluorescent antibody method.

2. Uterine and gastrointestinal lesions observed in hamsters inoculated with fluids from tissue cultures which had received preparations from human autopsy material from patients that had died of cancer.

From a total of 263 inoculated hamsters of both sexes that have been autopsied 171 were females. Of the 171 females 18 percent developed a hemorrhagic lesion of the uterus. In many of these there were macerated fetuses, several with extrusion of one or more fetus into the peritoneal cavity. Hemorrhagic lesions of the G.I. tract have also been observed in many of the hamsters of both sexes. At first it was believed to be of the same etiology as the uterine lesion, but it has also been found in numerous control animals. The uterine lesion has been seen in only 4 of 90 control female hamsters which have been autopsied.

A histologic study of these hemorrhagic lesions is in progress.

Part B included Yes

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Calender Year 1959

Part B. Honors, Awards, and Publications:

Publications other than abstracts from this project:

Periodicals:

Stewart, S. E., Eddy, B.E., Irwin, M., and Lee, S.: The development of resistance in mice to tumor induction by S E polyoma virus. Nature (in press).

Stewart, S. E., Eddy, B. E., and Stanton, M. F : Neoplasms in rodents induced by S E polyoma virus. Acta Un. Internat. contra cancrum 15: 842-885, 1959.

DiMayorca, G.A., Eddy, B. E., Stewart, S. E., Hunter, W. S., Friend, C and Benditch, A.: Isolation of infectious deoxyribonucleic acid from S E polyoma infected tissue cultures. I. A preliminary report. Proc. National Acad. Sc., December 1959 (communicated by Gilbert Dalldorf).

Stewart, S. E. and Eddy, B. E.: Tumor induction by S E polyoma virus and the inhibition of tumors by specific neutralizing antibodies. Am. J. Pub. Health 49: 1139-1145 1959.

Stewart, S. E., Eddy, B. E. and Stanton, M. F.: Induction of neoplasms in mice and other mammals by a tumor agent carried in tissue culture. Proc. Third Canadian Cancer Conference. 287-305, 1959.

Eddy, B. E., Stewart, S. E., and Stanton, M. F.: Induction of tumors in rats by tissue culture preparations of S E polyoma virus. J. Nat. Cancer Inst. 22: 161-171, 1959.

Eddy, B. E., and Stewart, S. E.: Physical properties and hemagglutinating and cytopathogenic effects of the S E polyoma virus. Proc. Third Canadian Cancer Conference. 307-324, 1959.

Eddy, B. E., Stewart, S. E., Warnshaw, R. L., and Young, R. O.: Induction of subcutaneous nodules in rabbits with the S E polyoma virus. Nature: 183: 766-767, 1959.

Eddy, B. E. and Stewart, S. E., Characteristics of the S E polyoma virus. Am. J. Pub. Health 49: 1486-1492, 1959.

Books:

Stewart, S. E.: Polyoma Virus, Section A. In Advances in Virus Research. Academic Press, New York (in press).

Stewart, S. E. and Eddy, B. E.: A Review on the Biological Properties of S E Polyoma Virus. In Monograph on Leukemia Symposium, [illegible] (In press.)

Serial No. NCI-404
1. Laboratory of Biology
2. Cellular Biology Section
3. Bethesda, Md.

PHS-431
Individual Project Report
Calendar Year 1959

Part A.

Project Title: DNA synthesis in normal and neoplastic tissues and cells. Synthesis of phosphorylated DNA precursors.

Principal Investigator: Ruth K. Kielley

Others Investigator: W. C. Schneider

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: 0

Project description:

Objectives: The long range objective of this study is to gain some understanding of enzymatic control mechanisms in DNA synthesis by which cell growth and multiplication are regulated. Recent findings on the nature of DNA precursors indicate that a study of the formation of deoxynucleotide triphosphates in normal and tumor cells might be feasible and worthwhile at this time.

The immediate objectives were concerned with (1) development of an assay method to detect and measure deoxynucleotide kinase activities in various kinds of normal and tumor cells and (2) development of improved methods for isolating nuclei in which various activities leading to DNA synthesis might be studied.

Methods: The assay method used by Kornberg for measuring deoxynucleotide kinase activity in extracts of E. coli was selected for this study. The method depends on the use of P^{32}-labeled deoxynucleotides which are converted to triphosphates by kinase action in the presence of ATP. The methods used for preparation of P^{32}-labeled deoxynucleotides were followed through. These included special methods for culturing E. coli in P^{32}, isolation and hydrolysis of labeled DNA to 5'-deoxynucleotides, separation of the individual nucleotides by ion exchange chromatography and identification by spectral characteristics.

Methods for isolating nuclei involved suspending the cells in various kinds of modified sucrose solutions, rupturing the cells by various mechanical means and separating the nuclear fraction by

differential centrifugation. The criterion used for evaluating the various conditions employed was based upon the morphological characteristics of the isolated nuclei under phase microscopy. Those conditions preserving nuclei in a state most nearly like that in the intact cell were considered desirable.

Principal findings: The technical problems involved in the preparation of deoxynucleotides from thymus and of very small amounts of P^{32}-labeled nucleotides from E. coli have been satisfactorily worked out. It has been found that in growing E.coli to a limit on a low phosphate medium for P^{32} incorporation, vigorous aeration such as is possible in a gas washing cylinder is necessary. The glassware need not be absolutely sterile, but an initially sterile medium and inoculum of a pure culture of E. coli Strain B are necessary to avoid large amounts of acid production in the medium by extraneous organisms which can cause growth to stop before the phosphate has been exhausted from the medium. At a phosphate concentration of 33 μ moles/100 ml. instead of 80 μ moles as generally used for P^{32} incorporation, the utilization of phosphate into DNA is more efficient, but this is offset by the larger volume of culture required to produce the same amount of DNA. No special difficulties were encountered in preparing DNA-nucleoprotein by alkaline digestion and precipitation with perchloric acid. In the hydrolysis of DNA after DNase digestion, by diesterase, it is necessary that the diesterase be free of 5'-nucleotidase. Large nucleotidase activities were found in the diesterase fractions obtained by acetone precipitation of snake venom after the lyophilized venom had been stored frozen for some time. The nucleotidase activity was destroyed by heating the diesterase solution for 20 minutes at 60° at pH 6.5 in the presence of serum albumin. The precipitate was centrifuged off and the clear supernatant solution was dialyzed overnight at 5° against 0.02 M Tris-HCl buffer, pH 8.8. The preparation has been kept frozen for a month without loss of activity. Nearly quantitative hydrolysis of E. coli DNA to 5'-deoxynucleotides was obtained by the combined action of DNase and diesterase. The individual nucleotides were separated by column chromatography on Dowex 1 -2x in the HCl-KCl solvent system. From a digest containing 11.6 μ moles of nucleotides, 1.8 μ moles of d-CMP, d-GMP and TMP were obtained. d-AMP was not recovered under conditions prevailing at room temperature.

The following method has been developed for isolating nuclei from ascites hepatoma cells.

Solution 1 - 0.25 M sucrose, 0.003 M $CaCl_2$, 5×10^{-4} M $MgCl_2$, 0.01 M histidine, pH 7.4.

Solution 2 - 0.25 M sucrose, 1×10^{-4} M $CaCl_2$, 2.5×10^{-4} M $MgCl_2$, 0.025 M histidine, pH 7.4.

One ml. of sedimented cells from 5 day transplants are washed twice with 5 volumes of solution 1, then resuspended to 10 ml. with solution 1. To this suspension, 0.02 g. of digitonin are added and the mixture is homogenized 2 minutes in a Potter-Elvehjem type homogenizer.

All operations are carried out in the cold (0° - 5°). The suspension is passed through a No. 22 hypodermic needle until most of the cells are broken and the nuclei for the most part are free of adhering cytoplasmic shreds. The number of passes through the needle depends on the particular cell preparation and the force used to push the suspension through the needle. Cell preparations from transplants much older than 5 days are much more resistant to breakage. Usually, for 5 day transplants, 5 to 10 passes are sufficient to break most of the cells. The broken cell suspension is centrifuged at 1600 rpm for 7 minutes, the supernatant solution is pipetted off and the nuclear sediment is resuspended to 10 ml. with solution 2. The suspension is again passed several times through the needle to remove cytoplasmic tags from nuclei and to break most of the remaining whole cells. After centrifugation, the nuclei are suspended in solution 2. By repeated washing by resuspension in solution 2, a nuclear preparation is obtained which is relatively clean. The nuclei in solution 2 are remarkably stable withstanding repeated washing and storage in the cold for many days. Analysis for cytochrome oxidase and ATPase activities showed 2 to 4 percent of the total homogenate activity present in the nuclear fraction. No evidence that these nuclei can support oxidative phosphorylation was found. It remains to be seen whether they possess activities in nucleic acid synthesis.

Modifications of the above procedure have been used to isolate nuclei from normal and regenerating rat liver and from Novikoff hepatoma cells.

Significance to cancer: A study of the enzymes involved in DNA synthesis in normal and tumor cells may help to provide a basis for understanding the nature of uncontrolled growth in cancer. Above all, the metabolic activities of the cancer cell appear to be geared to DNA synthesis as one of its principal activities.

Future program: The deoxynucleotide kinase activities of various types of normal and tumor cells will be investigated with the assay method now developed and attempts will be made to determine some of the conditions which influence these activities in cells.

Part B included: Yes No X

Serial No. NCI-406
1. Laboratory of Biology
2. Cellular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electron microscopy of normal and malignant cells, including studies on viruses.

Principal Investigator: A. J. Dalton

Other Investigators:

Cooperating Units:

Bryan (NCI-420)
Potter (NCI-427)
Moloney (NCI-410)
Manaker (NCI-416)
Belkin
Love
Sanford (NCI-428)
Stewart (NCI-433)
Merwin (NCI-414)

Man Years (Calendar year 1959): (Aug. 1- Dec. 1)
Total: .83
Professional: .5
Other: .33

Project Description:

(1) The study of plasma cell tumors in collaboration with Drs. Moloney and Merwin, begun with Dr. Potter in 1957, has continued without interruption during this interval. Material so far analyzed with the electron microscope includes two Pilgrim tumors, two tumors from Dr. Dunn, five obtained by Dr. Merwin and one developed by Dr. Potter. Among these, four were "spontaneous",

and six are considered to have been induced. Eight tumors arose in mice which presumably carried the mammary tumor agent. The other two arose in animals free of this agent. While variations in ultrastructural morphology were noted among these tumors, the differences could not be correlated with the differences in serum globulin patterns induced in mice carrying the tumors. A feature common to all tumors so far examined has been the presence of virus-like particles in the cytoplasm of the tumor cells. The particles were found to be formed on the membranes of the endoplasmic reticulum, the membrane forming the outer shell of the particle. It was thought at first that the presence of these particles could be correlated with the fact that all of the tumors arose in mice carrying the mammary tumor agent, but their presence in cells of tumors arising in mice free of the agent eliminate this possibility. Furthermore, bioassays on cell free extracts of several of these tumors can now be considered as having demonstrated the absence of the mammary tumor agent in the tumors tested. The significance of these particles is unknown at present, but their regular occurrence in plasma cell tumors and their absence from normal plasma cells suggests that they are related in some way to the neoplastic state of the plasma cells. In connection with this study a search is continuing for a source of human myeloma cells plentiful enough to allow analysis with the electron microscope. In addition, with Dr. Potter a study of the cytogenesis of plasma cells with the electron microscope is under way.

(2) With Drs. Moloney and Manaker a study is being carried out on the morphology of the Moloney leukemia agent. This agent has already been characterized tentatively, but further work is necessary before a definitive description can be given. In the material examined so far the particles considered to be the infective agent have not been particularly numerous but they have been identified in spleen, lymph node and thymus of infected animals. It is hoped that with the use of tissue culture and high potency tumors semi-quantitative studies may be carried out which should answer the question as to whether or not the particles are the infective units of the Moloney leukemia virus.

(3) With Dr. Sarah E. Stewart, tissue culture preparations of human amnion and mouse embryo cells which show cytopathogenic effects following exposure to cell-free extracts of human tumors are being examined for the possible presence of virus-like particles. To date three samples of material have been examined with essentially negative results.

(4) A study with Dr. Sanford has just been initiated which includes an analysis of a strain of tissue culture cells grown from salivary gland explants. These cells will be

studied following infection with the polyoma virus and with the mammary tumor agent. No results have been obtained to date.

(5) Dr. Belkin had previously found that S-37 ascites cells developed numerous cytoplasmic vacuoles on exposure to certain plant polysaccharides. It was considered of interest to determine the site of origin of these vacuoles which in turn might be a lead indicating the cell components acted upon by the polysaccharide. An electron microscopic analysis has been initiated with the aim of settling this point. Preliminary results indicate that the vacuoles which become visible with the light microscope following treatment with the polysaccharide actually are derived at least in part from pre-existing submicroscopic vacuoles present in untreated S-37 cells. Many of these pre-existing vacuoles contain virus-like particles in various stages of development and some of the enlarged vacuoles of the treated cells were found to contain virus-like particles showing morphologic evidence of degenerative change. It is hoped that future studies involving variations in length of exposure to and concentration of polysaccharide will determine whether or not these preliminary observations are consistent and significant and whether the action of the polysaccharides is specifically at the site of virus formation.

(6) Dr. Love has developed a technique involving staining with a methylene blue-molybdate solution which visualizes different components of the nucleus and cytoplasm depending upon the length of time cells are exposed to the solution. Since molybdenum is a relatively heavy metal, it was considered worthwhile to test whether these differences in staining reaction observable with the light microscope could be observed in greater detail and with more accuracy with the electron microscope. Material has been harvested for this study. However, no observations have been made to date.

(7) A program has been proposed which, in collaboration with Dr. Ray Bryan, would involve the screening of pellets of extracts of human tumors with the electron microscope to determine the presence or absence of virus-like particles in these tumors. The program envisages the use of the electron microscope as a primary screening tool in this study. The work involved would be well beyond the capabilities of the unit as it exists at present. The only way in which this program could be activated without adversely affecting other research would be to recruit additional personnel and to purchase more equipment. Further detailed discussion followed by administrative commitments regarding recruitment of technical help and purchase of additional equipment will be necessary before this program can be initiated.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Gatenby, J. B. and Dalton, A. J.: Spermiogenesis in Lumbricus herculeus. An electron microscope study. J. Biophys. Biochem. Cytol. 6: 45-52, 1959.

Copeland, D. E. and Dalton, A. J.: An association between mitochondria and the endoplasmic reticulum in cells of the pseudobranch gland of a Teleost. J. Biophys. Biochem. Cytol. 5: 395-396, 1959.

Woodside, G. L. and Dalton, A. J.: The ultrastructure of lung tissue from newborn and embryo mice. J. Ultrastructure Res. 2: 28-54, 1958.

Honors and Awards relating to this project:

President, New York Society of Electron Microscopists, 1959-60.

Serial No. NCI-407
1. Laboratory of Biology
2. Cellular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Deoxyribose-containing compounds in normal, regenerating and cancer tissues.

Principal Investigator: Walter C. Schneider

Other Investigators: Jean Rotherham

Cooperating Units: None

Man Years (calendar year 1959):
Total: 3
Professional: 2
Total: 1

Project Description:

Objectives: To isolate and identify the deoxyribonucleosides and deoxyribonucleotides present in normal and cancer tissues and to determine the metabolic pathways in which they are involved and their relationship to the synthesis of deoxyribonucleic acid (DNA).

Methods: The deoxyribosidic compounds are separated from tissue proteins and DNA by making cold acid extracts of the tissues. The tissues are removed under anesthesia and frozen immediately in liquid nitrogen to prevent autolytic changes.

The deoxyribosidic compounds present in the acid soluble extract are isolated using paper and column chromatography and paper electrophoresis. The isolated compounds are identified by means of absorption spectra, chromatographic and electrophoretic behavior, and colorimetric and microbiological reactions.

Routine assays of deoxyribosidic compounds were made with the organism, L. acidophilus R-26, which specifically requires these compounds for growth. Urine samples were collected daily in modified plastic cages, which were autoclaved each day. The urine samples were filtered and treated with perchloric acid prior to the measurement of deoxyribosidic compounds. The compounds present in the urine were purified chromatographically.

C^{14}-labeled deoxycytidine was prepared from commercial cytidine-2-C^{14} and deoxyinosine using a transdeoxyribosidase present in extracts of L. acidophilus R-26. The deoxycytidine-2-C^{14} formed was purified chromatographically and demonstrated to be homogeneous radiochemically and to consist entirely of deoxycytidine on the basis of microbiological activity.

Major Findings:

A. Excretion studies. Our main object in studying the urinary excretion of deoxyribosidic compounds was to determine whether these compounds come from the diet or intestinal flora and whether the animal can utilize them in situations where rapid DNA synthesis is necessary (presence of a tumor or regenerating liver). Although these studies have been complicated not only by technical difficulties as well as by variations in individual excretion patterns, the following tentative conclusions have been reached.

1. The deoxyribosidic compounds in the urine are not derived entirely from dietary constituents. This was shown by studies on fasted rats and on rats fed a chemically defined, soluble amino acid diet (kindly furnished by Dr. Milton Winitz).

2. These compounds are not produced by organisms in the intestinal tract, since they were excreted in the urine of germ free animals.

3. After partial hepatectomy, the excretion of deoxyribosidic compounds appears to decline and reaches a minimum level 2-3 days after the operation. Since this did not occur in rats which were laparotomized and implanted with a liver lobe from another animal, it would appear that during the period in which DNA is being rapidly synthesized in the liver the deoxyribosidic compounds normally excreted in the urine are diverted into synthetic pathways. Unfortunately, this excretion pattern was not observed in 100 percent of the hepatectomized rats studied and thus additional experiments will be necessary to determine why this was not true.

4. In the case of rats bearing transplants of the Novikoff hepatoma, the urinary excretion also appears to be decreased at a time when the tumor is growing most rapidly. Here again, however, there were exceptions to this conclusion.

5. Studies on the identity of the urinary compounds, although incomplete, indicate that the major compound present is deoxycytidine and that minor amounts of deoxyuridine and an unidentified nucleotide material are also excreted.

B. Isotopic studies. It was hoped that the identification of the deoxynucleotides present in tissues could be facilitated by the use of a specific precursor of DNA, deoxycytidine, labeled with C^{14}. Such experiments were also indicated by our previous findings that this pyrimidine nucleoside is found in many tissues, including tumors, as the major deoxyribosidic component. The use of this precursor could then be expected to elucidate the pathways involved in the synthesis of pyrimidine DNA nucleotides in a straightforward manner.

Attempts to use this precursor in vivo have been abandoned temporarily because the injected material was found to be almost uniformly distributed throughout the body of the animal. Thus the amount of radioactivity localized in a single tissue was too low to make isolation worthwhile. In order to circumvent this problem, the use of deoxycytidine with a much higher specific activity would be needed. For this purpose, deoxycytidine labeled with tritium was ordered from an outside contractor. After a six month delay we were informed that the manufacturer had been unable to prepare the material. However, the manufacturer was not the one we originally specified and the material is being reordered.

In the meantime, several in vitro experiments have been made using labeled deoxycytidine. Suspensions of a C3H ascites hepatoma were incubated with the labeled material and the products isolated and identified. The DNA of the cells became labeled progressively during these experiments. In the first experiments, the cells were incubated in the ascitic fluid because this was thought to be more physiological. However, the suspensions rapidly became acid and the radioactive products formed indicated that degradation was occurring.

It was found that a more suitable incubation medium consisted of a buffered isotonic salts solution. In this medium, the main product formed was the nucleoside, deoxyuridine. Thymidine and uracil were also formed but at a level one-fifth as great. The specific activity of the compounds isolated showed that the deoxycytidine added and the deoxyuridine formed became progressively more dilute during the incubation. This indicated that there is an endogenous source of deoxycytidine in these cells.

Although only small amounts of radioactivity were found in the nucleotide fraction of these cells, it was sufficient to isolate and purify these compounds. These studies, although incomplete, indicate that there may be 14 or more deoxynucleotides present. The spectra of these compounds indicate that 6 contain deoxycytidine, 1 deoxyuridine and 1 thymidine. The rest have not been sufficiently purified to obtain spectra.

C. Function of deoxycytidine diphosphocholine (dCDP-C) and deoxycytidine diphosphoethanolamine (dCDP-E). In order to study the function of these compounds, which were isolated last year from the Novikoff hepatoma, it is necessary to have radioactive preparations of these compounds. Such preparations would also be very useful in determining the presence and concentration of these compounds in other

tissues. In collaboration with Dr. John Weisburger, the synthesis of phosphoethanolamine-1,2-C^{14} (PEA) has been completed and the synthesis of the phosphocholine (PC) analog is in progress. The synthesis of deoxycytidine triphosphate (dCTP) (unlabeled) has also been completed. Since these compounds are now available, the enzymatic synthesis of dCDP-C and dCDP-E by normal and tumor tissues can now begin.

The chemical synthesis of the latter compounds has never been very satisfactory due to low yields and side reactions. In order to make full use of the radioactive materials, some of the newer methods of Khorana, by means of which these compounds could be made, are being investigated. The results to date are inconclusive.

Significance to cancer research: The fact that the synthesis of DNA is one of the obligatory reactions in the production of new cells, both normal and malignant, has focused the attention of more and more investigators on the study of this unique cellular material. The elucidation of the reactions by which DNA is formed is thus considered to be one of the most important problems in cancer research today.

The experiments described above are highly relevant to these studies. The urinary excretion studies suggest that tumors and other growing tissues may call upon the deoxycytidine present in the tissues and circulating in the blood as a building block for DNA synthesis. The in vitro experiments show that at least one tumor cell is capable of making its own deoxycytidine. Whether this capability is shared by other cells or whether this ability fulfills the requirements of the tumor cell is not known. These are, however, important questions which must be resolved before the control of DNA synthesis, and hence, of cell multiplication, can be satisfactorily approached. The identity of the deoxynucleotides present in the acid soluble extracts of tissues is also important from this standpoint. It should be mentioned that of the pyrimidine deoxynucleotides now known, 5 contain deoxycytidine, 3, deoxyuridine and 3, thymidine. This suggests that there may be some new deoxynucleotides in the hepatoma since the isotopic experiments indicated the presence of 14 components.

Proposed course: The urinary excretion studies will be continued. It is hoped that during the next year it will be possible to resolve some of the inconsistencies that have been observed and to decide whether the animal can, in reality, use endogenous deoxyribosidic compounds for DNA synthesis in tumors and regenerating liver.

The work on the purification of the radioactive nucleotides will continue. Besides the determination of the identity of these compounds, it will be important to know the specific activity of these compounds because the latter will enable a decision to be made as to the utilization of the compounds in the synthesis of DNA.

Both the chemical and enzymatic synthesis of dCDP-C and dCDP-E will be studied. The enzymatic formation of these compounds is especially important because their presence in the hepatoma indicates that the tumor has a better mechanism for their formation than does normal liver, from which they appear to be absent or present in much lower concentration. The pathways by which these compounds are utilized will also be studied to decide whether the presence of these compounds in the tumor is due to overproduction or inefficient utilization.

Part B included: Yes X

PHS-431
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Schneider, Walter C.: Mitochondrial metabolism. In Advances in Enzymology 21: 1-72, F. F. Nord, ed. Interscience Publishers, New York, 1959.

Serial No. NCI-408
1. Laboratory of Biology
2. Cellular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on lymphocytic tumors

Principal Investigator: Emma Shelton

Other Investigators: Mary Rice

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2.5
Professional 2
Other: .5

Project Description:

Objectives: To study the physiology and morphology of lymphocytic tumor cells and their relationship to the normal cells of the host animal. The behavior of the tumor cells is an integration of biological factors such as, growth rate, locomotion rate, metabolism, immunological properties, and the integration of these factors must be considered against the background of host influence. It is the purpose of these studies to attempt to sort out and examine separately some of these factors in order to assess more properly the relationship between tumor cells and the normal cells of the host.

Methods: The basic biological tool used in these studies is the ascites tumor. The free cells of the peritoneal fluid of the mouse were studied as representing the normal tissue that is in contact with the ascites cells.

Specific methods: (1) Double diffusion chamber: For details of construction and use, see 1957 annual report. The cells and fluid in the diffusion chamber were analyzed by--(2) Histochemical methods--for connective tissue and elements making up the intracellular matrix; (3) Paper chromatography--for hydroxyproline, the unique constituent of collagen, (4) Electron microscopy--of collagen fibers and cell ultrastructure.

Results:

1. Growth and modulation of normal cells in diffusion chambers: In seeking to obtain concrete information about the behavior of normal cells in the presence of tumor cells, the behavior of normal cells alone was studied. In so doing we found ourselves drawn into a 60-year-old polemic--reluctantly at first and then with enthusiasm. We believe that we have the means at hand to solve conclusively the problem of the fate and function of the free cells of the reticulo-endothelial system.

The diffusion chamber technique has provided new evidence in support of the lymphocyte-to-fibroblast transformation. The technique has permitted us (a) to grow cells of known number and type for long periods of time in an isologous or heterologous environment, (b) to examine cell relationships in stained, whole-mount preparations, and (c) to analyze chemically the contents of the chambers. When the free cells of the peritoneal fluid of mice are grown in diffusion chambers, transformations occur which lead to the formation of an organized tissue. The lymphocyte modulates into an indifferent cell which, in turn, has the capacity to become a fibroblast or plasma cell. The macrophage also appears to have the same capacity. Granulocytopoiesis occurs in the chambers and mast cells are still present after 140 days. Collagen was identified in the chambers after the cells had grown for two weeks and the collagen content of the chambers continued to increase with time.

There is reason to assume that in the animal the free cells of the reticulo-endothelial system are responsive to the ever-changing environment that they encounter by virtue of their mobility, and that they would respond to the abnormal environment created by a wound, a tumor, infection, or metabolic disturbance in a manner not unlike that observed in the diffusion chamber.

2. Further studies on the penetration of lymphocytic tumor cells through cellulose membrane filters: In previously reported work, it was stated that tumor cells were not able to penetrate cellulose membrane filters (Type AA) having an average pore size of 0.8 u. More recent studies have caused us to revise this statement.

The cells of the highly invasive L1210 tumor penetrated the AA filter 100 percent of the time after 30 days' growth in the chamber (40 chambers) and established a visible amount of growth on the "non-cell side" of the chamber. The relatively non-invasive tumor, L#1, was not able to establish visible growth on the non-cell side of the chambers by 120 days (40 chambers) but in one chamber L#1 cells were microscopically visible on the non-cell side of the stained center filter. Normal cells introduced into the chambers with the L#1 tumor cells were able to penetrate the center filter and were found on the non-cell side of the chambers. In addition, there were clumps of rounded-up cell ghosts found consistently, these were probably dead tumor cells.

The results demonstrate that two tumor populations, given an equal chance, may or may not be able to penetrate an artificial barrier and establish growth at a distant site. This difference in the capacity of cell populations to survive may operate in aiding or hindering the establishment of new sites of growth by these tumors while they are growing in the host.

3. Behavior of tumor cells when grown on explants of connective tissue in diffusion chambers: We have reported previously that normal peritoneal cells behaved differently in the diffusion chamber according to the tumor that was grown with them. In the case of the "invasive" tumors L#2 and L1210, the normal cells and tumor cells appeared to grow independently, but in the case of the "non-invasive" tumors L#1 and P353, there was a definite association between the two cell types in which the tumor cells grew in definite clumps that were surrounded and enclosed by the normal cells.

We have followed up this observation by growing L1210 and L#1 together with explants of normal connective in the same chamber. The fibroblasts of the subcutaneous fat pads of the mouse grow luxuriantly in the diffusion chamber. By introducing ascites cells into the same chamber with the fibroblasts, the relationships between the two cell types could be studied. The results of this experiment were striking. Examination of the stained filters on which the cells grew showed that there was absolutely no association between the invasive L1210 cells and the connective tissue fibroblasts. The tumor cells grew as free floating, independent entities. The cells of the non-invasive L#1 grew in intimate association with the connective tissue fibroblasts. When the edge of the fibroblastic growth stopped, the tumor cell growth also stopped. In many places the advancing edge of the growing connective tissue sheet was ahead of the tumor cell growth. We hope to explore further the reasons for this extraordinary behavior.

Significance to cancer research: The lymphocytic leukemias in mice very closely resemble the lymphocytic leukemias in man. The further elucidation of the characteristics of the disease in mice should have a direct bearing upon the disease in man.

Proposed course of the project: (1) Investigation of the relationship between normal cells and malignant lymphocytes grown in diffusion chambers will continue. Specifically we would like to isolate and grow as pure lines the normal lymphocyte, macrophage, polymorphonuclear leucocyte, and the mast cells. We would like to isolate and grow tumor cells that are uncontaminated by normal cells. The chambers are constructed in such a way that it is possible to isolate the cells from some of its products. We hope to learn something of the cells' metabolism by studying its products.

The objectives outlined in (1) will probably keep us busy during the coming year but if we have time and space, one or several of the following lines will be pursued.

(2) Speed and mode of locomotion of normal and malignant lymphocytes.

(3) Electron microscope study of relationships between the malignant lymphocyte and cells of the invaded tissue.

(4) A study of the surface charge of normal and malignant lymphocytes by observation of their mobility in an electric field.

(5) Electron microscopy of collagen production and the effect thereon of the presence of tumor cells.

Part B included: Yes X

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Shelton, E. and Rice, M. E.: Relation of invasive capacity to passage of lymphocytic cells through cellulose membrane filters. Nature 184: 479-480, 1959.

Shelton, E. and Dalton, A. J.: Electron microscopy of emperipolesis. Jour. Biophys. and Biochem. Cytology 6: December 1959.

Serial No. NCI-409
1. Laboratory of Biology
2. Cellular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electron microscope studies on various normal, virus associated and malignant cells.

Principal Investigator: Robert F. Zeigel

Other Investigators:

M. F. Stanton
R. A. Manaker (NCI-416)
W. R. Bryan (NCI-420)
A. N. Weinberg
R. W. Mendler

Cooperating Units:

NCI; Laboratory of Biology, Laboratoryof Biochemistry, Laboratory of Clinical Pharmacology; NIAMD: Clinical Endocrinology Branch; NHI: Laboratory of Cellular Physiology and Metabolism.

Man Years (calendar year 1959):
Total: 1 1/3
Professional: 2/3
Other: 1/2

Project Description:

Part one. A major portion of our time and effort was spent in preparing and examining tissue from the Bovine Ocular Squamous carcinoma. This study began with the collection of the tissue from Hereford cows at Bay Manor Farm in Lewes, Del. and from the Walter Reed experimental farm in Maryland. The cooperation of the ranch foreman and his staff was greatly appreciated. The tissue was fixed, embedded and examined. Portions of the

tissue were taken for tissue culture work by Dr. Manaker and for the concentration of the microsomal and possible virus fraction by Dr. W. R. Bryan. Parts of the tissue which presumably represented the early plaques in the highly keritinized epithelium were stored in glycerine for a period of time before fixation. This treatment is reported to aid in the preparation of this difficult tissue for sectioning while preserving any viable virus particles which might be present. After a considerable amount of microscopy was performed it was decided that in the particular tissue examined particles with the morphology of viruses were not present and that further efforts to visualize them were not warranted.

Part two involved the study of virus production in pancreatic acinar cells of a young chick. Previous observations on the virus particles concerned with lymphomatosis and the Rous virus indicate a very different pattern of formation. The observations made in this study indicate the possibility that many diverse tissues may be concerned with the patterns of virus behavior and reproductive cycle. Much more work has yet to be done to elucidate this problem. However, a brief paper is in the process of being written to document this finding.

Part three, now under way, is an attempt to rework, with more up to date techniques, a study begun by Dr. Dalton and Dr. R. Kendler on the albumen glands of the chicken oviduct. Staining techniques should enhance and aid in the localization and identification of active regions of the cells. Hopefully, this study will contribute to a better understanding of the secretory cycle of this copiously produced proteinaceous substance.

Part four is a preliminary study concerning the effect produced by the incubation in various concentration of insulin on fatty tissue from a diabetic patient. It appears that increments in the titers of insulin produce an increase in the number and size of pinocytotic-like vesicles in the

membranes and cytoplasm of the fat cells. It is hoped that further studies may confirm the hypothesis that, in part at least, insulin plays some role in allowing blood sugar to enter the various cells of the body, presumably as a membrane effect on the end organ. This study was begun with Dr. A. Weinberg.

Part five concerns some preliminary observations on millipore-filter chambered cells of studies organized by Dr. E. Shelton. This approach should be a fertile one from the point of view of elucidating the details of cyto-differentiation of some of the cells of the hemopoietic series. This work is being held in abeyance at present.

Part six. In a comprehensive study of microsomal fractions of Novikoff Hepatoma cells, a co-authorship in a paper with Dr. E. Kuff has resulted. A large number of variously prepared specimens were examined and photographed. It is believed that some insight into the basic building blocks of cytology may be gained through this and similar studies. This type of study, previously pursued by Dr. Kuff and Dr. Dalton and members of the Section, is basic to the ultimate concept of cellular morphology and physiology. It is at this level of endeavor that these two terms become virtually synonomous.

Part seven involved the testing and standardization of a new lead hydroxide staining technique suggested by Dr. Dalton. A short note for publication resulted.

Part eight is concerned with the development of a doctorate thesis, parts of which are being prepared for publication.

Part nine. During Dr. Dalton's absence considerable time and energy was spent in supervising and attempting to coordinate the installation of a new electron microscope and other laboratory equipment which should facilitate and aid the working conditions of the laboratory.

Part B included Yes No X

1. Laboratory of Biology
2. Cellular Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project title: Diffusion chamber and transparent chamber research

Principal Investigators: Ruth M. Merwin

Other Investigators: Thelma Dunn

Cooperating Units: None

Man Years (calendar year 1959):
Total: 4
Professional: 2
Others: 2

Project description:

Problem 1--Induction of plasma-cell tumors: Ruth Merwin and Thelma Dunn

Objectives: The object of these studies was to determine why plasma-cell tumors arose in BALB/c mice carrying intraperitoneal diffusion chambers which contained C3H mammary-tumor tissue. Experiments were set up to try to find out which of the many factors present were essential for the induction of the tumors. Such factors as the following were considered: the chamber itself, the homologous and tumorous nature of the tissue within the chamber, the presence of the mammary tumor agent in the tissue in the chamber. (Dr. Dalton found virus-like particles in the induced plasma-cell tumors suggesting that a virus might be involved in the tumor induction).

Methods: Diffusion chambers were implanted as blanks or containing various types of tissues. Methods that would extract and concentrate any virus particles present in the tumor cells were employed to obtain preparations which were assayed for the presence of virus by injecting them into newborn mice.

Major findings: The most important finding so far is that a blank chamber can induce a plasma cell tumor. This finding makes it seem less likely that a virus plays an important role in inducing the tumors. (See reports by Drs. Fahey and Potter on studies made with these induced plasma cell tumors).

Proposed course: To determine why the blank chambers induce the tumors. The possibilities considered are that the chamber or some part of it may act as an adjuvant or as an antigen or both or that the chamber may act as an irritant or that the type of carcinogenic stimulus causing the sarcomas, which also arise in these experiments, may affect the plasma cells.

Significance to cancer research: These tumors are similar to multiple myeloma tumors in man. One of the tumors is of particular interest because it causes an abnormal protein to appear in the urine just as certain human myeloma tumors do.

Problem 2--Antigenicity of plasma cell tumor tissue in isologous hosts.

Objective: The object of these experiments was to find out whether plasma cells are antigenic in mice of the strain of origin of the tumor. This seemed a possibility since in C3H mice the tumors do not take in 100 percent of the hosts and since virus-like particles have been observed in the cells and also since the cells form an abnormal protein.

Methods: Diffusion chambers containing plasma cells were placed in hyperimmune hosts.

Major findings: The one line of malignant plasma cells studied (that is 7029) was antigenic in mice of the strain of origin.

Significance to cancer research: This antigenic difference between host and tumor might be exploited in trying to find a method for controlling the growth of this type of tumor.

Proposed course: To repeat the test using plasma cell tumors that have arisen in the experiments reported in the first problem. Preliminary experiments indicated that a good ascites form of the tumors is needed and these are being developed.

Problem 3--Virus studies.

Objective: It seemed possible that tumor viruses might be more infective when tissue containing them was placed in diffusion chambers. The virus in the chamber might be less affected by host reactions (just as heterologous tissue within the chambers survives much longer than when placed directly in contact with host tissues) and that the chamber might thus serve as a long continued source of virus.

Methods: Diffusion chambers were used.

Major findings: The mammary tumor agent does not seem to be any more infective when inside the diffusion chamber than when an equal amount of infected tissue is placed in an open chamber.

Significance to cancer research: If viruses were more infective when placed in chambers it might provide a better method than those available for tumor-virus assay.

Proposed course: To see whether other tumor inducing viruses act like the mammary tumor agent.

Problem 4.--To study the effect of local irradiation on x-ray resistant tumors.

Objectives: The immediate objective is to test the sarcomas arising in the experiments described in problem 1 to see if one can be found that will be radio-resistant when it is still small enough to be observed in transparent chambers. Sarcomas tested previously have been found to be radiosensitive when they are small although resistant when larger.

Methods: Tumors in transparent chambers were observed.

Major findings: None.

Significance to cancer research: To help in understanding the interaction between radio-resistant tumor cells and relatively radio-sensitive host blood vessels in the response of certain tumors to treatment with x-irradiation.

Part B included: Yes X

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications.

Publications:

Marvin, R. M. and Algire, G. H.: Induction of plasma-cell neoplasms and fibrosarcomas in BALB/c mice carrying diffusion chambers. Proc. Soc. Exper. Biol. Med. 101: 437-439, 1959.

Serial No. NCI-417
1. Laboratory of Biology
2. General Biology Section
3. Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigation of the role of genes and their relationship to non-genetic factors in the development of cancer.

Principal Investigator: W. E. Heston

Other Investigators: Margaret K. Deringer, George Vlahakis, and Arnold W. Pratt

Cooperating Units: NCI-421

Man Years (calendar year 1959):
Total: 6
Professional: 2
Other: 4

Project Description:

Objectives: To ascertain the role of genes in the development of tumors in mice and to relate the genetic effects with those of other biological, chemical or physical agents in the causation of cancer in mice. To identify and locate specific genes influencing the occurrence of the various kinds of tumors and to determine the gene-action pathways through which their effects become manifest.

Methods: We continue to use mice exclusively as our experimental animals. We are carrying 8 strains including C3H--a high mammary-tumor and high hepatoma strain, C3Bf--like C3H but without the mammary-tumor agent, A--high lung tumor and high mammary tumor strain, YBR--that carries the lethal yellow gene, C57BL and C57L--low mammary-tumor and low lung-tumor strains, IC--the strain with very large body size, and Al--the strain that Dr. Tsubura brought from Japan and that has a relatively high incidence of mammary tumors. In addition we have the mutant stock Vob that carries the recessive gene obese and also carries piebald.

Breeding techniques are used to produce desired hybrid generations in which segregation of the rather interesting genes under study can be observed, and the occurrence of tumors is observed in the various segregants. All animals are autopsied. In the mammary tumor work the animals are killed after the mammary tumor has appeared, but in the pulmonary tumor work and some of the studies on hepatoma the animals are killed at specific ages. All unusual tumors and all mammary tumors arising in the absence of the mammary tumor agent are

checked with histologic sections, but unquestionable mammary tumors, hepatomas and pulmonary tumors are not routinely sectioned since this would overtax the sectioning unit. The pulmonary tumors are routinely counted under the dissecting microscope after the lungs have been inflated with fixative. Foster-nursing is employed to add or eliminate the mammary tumor agent, and carcinogens are injected or fed in appropriate dispersions or solutions. In the oxygen studies exposures have been carried out in metabolic chambers especially designed by Dr. Arnold W. Pratt. Data are analyzed with acceptable tests for significance and correlation procedures.

Major Findings: The rather extensive project to study further the genetic control of the mammary tumor agent or virus in crosses between C3H and C57BL has been completed and the results presented for publication. Gene control over the propagation of the agent was confirmed, but the segregation data did not support an interpretation of single gene control, but suggested that the agent can be propagated in the presence of one or more of several genes. The segregation of these genes as revealed in the occurrence of mammary tumors in appropriate foster-nursed test C3HF and C3He females was not apparent in the first backcross where it might have been expected but instead in the second backcross. Thus, a female lacking any of the genes for propagating the agent apparently does not eliminate the agent but there is no multiplication of the agent and through dilution it is lost in subsequent generations. There was evidence that females that do propagate and transmit the agent can vary in the quality or quantity of the agent produced presumably depending upon the number they have of the genes for propagating the agent. Tumors other than mammary tumors were also tabulated. Of these, hepatomas were the most frequently occurring tumors in the C3HF and C3He test groups, and reticulum cell tumors were the most frequently occurring tumors of the hybrid groups. Reticulum cell tumors occurred in as high as 26 percent of the second backcross group.

The study on the effect of urethan on hepatomas in males of strains C3H, C3HF, and C3He mice was completed during the year, and the results were accepted for publication. A surprisingly high incidence of hepatomas was found in these three strains, and the occurrence of the tumors was significantly increased with urethan. Other tumors or lesions induced with the urethan were pulmonary tumors and hemorrhagic lesions some of which were evidently hemangioendotheliomas in the liver. These hemorrhagic lesions sometimes do, however, occur without urethan. The increase in "spontaneous" hepatomas that we have observed in our C3H lines has undoubtedly been due in part to a genetic change in the strain. Part of the increase, however, has been due to a change in diet. C3HF males fed Purina laboratory Chow had a significantly lower occurrence of hepatomas than did those fed the NCI pellets, the diet we now routinely feed to our colony. In addition to this we have also recorded greater growth with the NCI pellets than with the Purina food. The presence or absence of the mammary tumor agent had no effect upon the occurrence of the hepatomas.

Further results have been obtained on the effects of specific genes on pulmonary tumors, mammary gland tumors and hepatomas. All of the C3H F$_1$ breeding and virgin females for studying the effect of the lethal yellow gene on mammary tumors have now been autopsied. There was very little difference between the yellow and the agouti breeders. Of the 106 yellow breeding females 98 percent developed mammary tumors at an average age of 7.9 months, and of the 106 agouti breeding females 97 percent developed mammary tumors at an average age of 8.4 months. In the virgin females there was very little difference in incidence of tumors, but the tumors came up at a much later age in the agouti females. Of the 88 yellow virgin females 100 percent developed mammary tumors at an average age of 8.1 months, and of the 111 agouti virgin females 96 percent developed mammary tumors at an average age of 15.2 months. Thus, it appears that the difference caused by this gene is basically a hormonal difference which can be seen in the virgin females, but this hormonal difference is obliterated by breeding the females. It should be noted that these data confirm Little's earlier observation that the yellow females had their tumors earlier, but do not confirm his observation that the final incidence in the yellows was lower than that in the non-yellows which had always seemed improbable to us. The fact that approximately 100 percent of the females of all groups in our experiment had tumors indicates the good health maintained in our animals, and this may in part account for the difference between the two experiments.

Data collected during the year indicate a positive effect of the lethal yellow gene also on the occurrence of spontaneous hepatomas. This has been shown in F$_2$ hybrid males from crosses between strains C3H and YBR. Preliminary calculations were made only on the data from males that lived to the 16 months of age set for autopsy. Of the 53 yellow males 100 percent had hepatomas, whereas of the 94 agouti males 88 percent had hepatomas. An even greater difference was shown in the average number of hepatomas per animal. The yellow males averaged 3 hepatomas per animal and the agouti males averaged only 2.02. It has been known that the lethal yellow gene increases body weight and it did in this experiment. In addition total body length and length of the femur were measured and the gastrocnemius muscle was weighed. In animals of this age the muscle weights were not satisfactory for correlation analyses but the skeletal measurements were. In the combined groups there was a positive correlation between occurrence of hepatomas and each of the three measurements, body weight, body length, and length of femur.

Thus the lethal yellow gene of the mouse has been shown to increase the occurrence of pulmonary tumors, mammary tumors, and hepatomas. These data extend the suggestion that many of these specific genes effecting occurrence of the various tumors do so through processes also related to animal growth as measured in skeletal and muscular development but not as measured in body weight greatly effected by adipose tissue.

The study of the effect of oxygen tension on induction of pulmonary tumors in strain A mice done in cooperation with Dr. Arnold W. Pratt has been somewhat retarded owing to shortage of strain A mice. However, some progress has been made during the year in dose-response studies. We have found almost a perfect straight line relationship between number of induced pulmonary tumors and concentration of oxygen to which the animals were exposed for the four concentrations, 100 percent, 40 percent, 21 percent (air), and 8 percent.

Significance to the program of the Institute: This program is contributing toward a more thorough understanding of the etiology of cancer. The program emphasizes the importance of genes in etiology even to the extent of measuring effects of specific genes upon a number of different types of cancer. The program also emphasizes the relationship of genes to other known factors in the etiology of cancer. It emphasizes that in studying the effects of viruses on the induction of cancer the genes controlling the propagation of the viruses should also be considered. The program is helping to tie the effects of specific genes on specific types of cancer to hormonal effects and to normal parts of the viral and cellular biochemistry - or basically to protein synthesis. The results from the oxygen studies help to tie the cancer process to metabolism. All of these contribute toward a broader understanding of the formation of cancer - the approach to the prevention of cancer - and this must be the ultimate aim of the Institute.

Proposed course of the project: Some progress has been made in this laboratory on the identification and analysis of the effects that specific genes identified as specific susceptibility genes on various types of neoplasms than has been made elsewhere, and it is planned for this line of work to be continued. Additional work now in progress includes studies of the effect of the lethal yellow gene on inbred hepatomas in addition to the spontaneous hepatomas [illegible] tests for the effect of the gene obese on spontaneous pulmonary tumors, on spontaneous and inbred hepatomas, and on mammary tumors are in progress. Studies are also underway on occurrence of pulmonary tumors in F_1 and F_2 hybrids between strain A and strain AL mice have been selected for large body size, to see if there is a positive correlation between occurrence of pulmonary tumors and inbred genes influenced by these multiple and possibly non-specific genes for growth. We have recently obtained a NW stock of mice carrying the dwarf gene and we plan to test this gene upon a number of tumors.

The experiment for studying the possible [illegible] the lethal yellow gene increases pulmonary tumors [illegible] have to prepare to test. We shall use [illegible] that the gene increases normal growth rate [illegible] to the effect on tumors, and we will [illegible] to the [illegible] effect [illegible] to [illegible] by [illegible] [illegible] [illegible]

NCI diet the difference in body weight of the yellow and the brown controls is not great so we are having to repeat the study using Purina laboratory chow that does result in a remarkable weight difference.

We now have strain dd mice that Dr. Tsubura left with us when he returned to Japan. This strain came from Japan and is unrelated to any of the strains in this country. However, the females of the strain have a relatively high incidence of mammary tumors. The strain is now in the F_{10} generation of brother X sister inbreeding, and we plan to continue to inbreed the strain, to determine more accurately its incidence of mammary tumors, to see if it has the mammary tumor agent, and if so how the agent compares with that in our other strains.

In the studies on the effects of oxygen on pulmonary tumors we plan to continue the study of dose response and to look further into the question of whether or not there is a damaging effect of the high concentration of oxygen on the pulmonary tissue.

Part B included: Yes X

Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Vlahakis, George, and Heston, W. E.: Relationship between recessive obesity and induced pulmonary tumors in mice. J. Hered. July-Aug. 1959.

2. [illegible], W. E., Vlahakis, G. and Deringer, M. K.: High incidence of spontaneous hepatomas and the increase of this incidence with urethan in C3H, C3HF, and C3Ne male mice. J. Nat. Cancer Inst. 24: (Feb. 1960).

3. Heston, W. E., Vlahakis, G., and Deringer, M. K.: Delayed effect of genetic segregation on the transmission of the mammary tumor agent in mice. J. Nat. Cancer Inst. 24: (March 1960).

4. Heston, W. E.: Site of Gene Action and Carcinogenesis. Monograph on Genetics and Cancer published by M. D. Anderson Hospital and Tumor Institute. 1959.

5. Heston, W. E.: The impact of genetics upon cancer research. Bull. Roswell Park Memorial Institute. 1959.

6. Heston, W. E.: Review of the book Canadian Cancer Conference III. A.I.B.S. Bulletin, 1959.

Serial No. NCI-428
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Host-Tumor Relationships

Principal Investigator: Morris K. Barrett

Other Investigators: Malcolm S. Mallroy, Edward J. Breyere, and Margaret K. Deringer (in collaboration)

Cooperating Units: NCI-421
NCI-430
NIAID-25 (Dr. DuBuy)

Man Years (calendar year 1959):
Total: 5
Professional: 3
Other: 2

Project Description:

Objective: Our working hypothesis [illegible] field presently called "immunogenetics" which covers an area of overlap between the two classical disciplines but seems to differ significantly from each. This is a relatively new field and many borderlines and concepts are hazy, but appears to touch upon several obscure areas in biology, e.g. adaptive enzyme formation, embryonic induction, differentiation, irradiation protection, and the broad but ill defined field of "tumor immunity." It is becoming increasingly apparent that biological interactions involve some undiscovered "information" or "recognition" system of general application. Our hypothesis assumes that some such system is involved in the intercellular reactions of normal differentiation, growth, physiology and that the abnormal differentiation and growth in cancer represent an aberration in such a system. We also entertain the notion that such a breakdown may not always be complete and that therein may lie the explanation of spontaneous regression of cancer, temporary remissions of cancer, and the apparent control of metastasis and recurrence.

More specifically, we are attempting to discover what "tissue immunity" and "transplantation antigens" are. At first thought they may appear to be rather nonsensical laboratory playthings with no real place in natural phenomena. However, they are so widespread in nature that, speaking teleologically, they must have some physiological function. We believe that discovery of the true nature of the "antigen" and the "immunity" in this field will open a new approach to many problems in cancer, both as to the intrinsic nature of neoplasia and as to its control.

Methods: The basic procedure is transplantation of standardized tumors, or skin, into hosts of known, but highly varied genetic constitution and observing the effects of experimental variation of hosts, or tumors, or both and of immunizing injections, treatment of the antigens, effects of chemical or physical agents, etc. The disciplinary techniques are those of genetics, immunology, and experimental surgery.

Major Findings: In relationship to what is variously called "adaptation" of a tumor, or the "F_1 hybrid phenomenon," or the "Barrett-Deringer phenomenon," none of which is a good term, there have been no major positive findings to change viewpoints previously expressed. Another tumor "adapted" but only poorly and was dropped because of technical difficulty. Another attempt to learn whether simultaneously implanted "strait" and "adapted" tumors interact yielded unconvincing data that, taken with previous data, leaves the question open. Another attempt to see if hybrid tumor adapts gave a negative result on poor data; thus weak data have pointed both directions. (This has a bearing on "dosage" effects). With respect to a hybrid fetus we have observed no evidence of a reaction to the pure strain mother - but the mother is changed, as will be mentioned later.

Over a period of years there have been several indications that the Histocompatibility-2 (H-2) complex of antigens might have some special features with respect to these phenomena. Our outcrosses involved differences at this strong locus but the results generally suggested that whatever transplantability changes occurred did not involve changes in this antigen complex. In some ways this seems odd. We are in the early stages of work with new tumors. In this case the tumors and animals are all alike at this locus. The thought is that if changes occur they must be based on other loci. One of the tumors is now growing in about 50% of the foreign strain but the results suggest that what we are getting is "immunoselection" and not "adaptation." If this is so we will have an interesting opportunity to try the effect of other outcrosses on the same tumors. This might throw some light upon the relation of "the hybrid phenomenon" to "somatic mutation," "transduction," and the like.

We have had a long standing interest in the "individuality" of tumors. The varied behavior mentioned above is one aspect of this. Another is our tentative observation that in some combinations tumors may not, as is generally believed, grow as well in the F_1 hybrid as in the parent strain. We think that we have seen this 3 times. If it is true it may require some revision of the simple concept of "one gene--one antigen," with "complete dominance." Along with this we have made careful, frequent measurements of a number of spontaneous mammary tumors in mice. The expected extreme variation in growth was observed among mice but those variations were no greater than those between multiple tumors on a single mouse. The latter can hardly be caused by the hormonal or metabolic status of the host.

With respect to the question of whether "adaptation" involves the introduction into the cell of something like DNA, protein, a virus, a plasmagene, or the like; we have been cooperating with Dr. Bailey in a small project. He has a technique for smoothing such material on very fine ground glass particles and then propelling it [illegible]

a cell grown in the test-tube. The idea is to extract possible tumor factors from one strain, inject them into cells of another strain, and see if the cells are changed in their interaction with the first strain. So far there has been much technical difficulty and few results but those obtained encourage persistence.

All the above involves study of the interaction of a homograft and its host. We have long been interested in the fact that in random breeding populations a fetus is a homograft but its interaction with the host seems to be exceptional. (This is also suggested in chorio-epithelioma.) To study this several combinations of in-strain and outcross matings of mice were set up, as reported last year; the mothers and offspring were observed for possible effects. The very interesting outcome may be epitomized as follows. Among females of a given strain those parous (not pregnant) by a male of a different strain exhibit "tolerance" toward a tumor of the second strain. The tolerance is specific for the strain of the male and is not seen in females parous by a third strain. Tolerance can be observed either as an increased number of takes of tumor in unimmunized females or as weak resistance in immunized females. The effect appears to increase with multiparity. A manuscript reporting this is in press. Further experiments in progress have indicated that the tolerance can also be seen as an increased survival of skin homografts. That, when a tumor of the third strain is used the specificity shifts to that strain. In the case of the latter tumor there may be a bizarre host-tumor relationship. A few observations suggest that there is some sort of migration of the tissue. The implant shows out as a localized mass; after attaining a moderate size it becomes cauliflower (not ulcerated) and may grow as a large circle or crescent with no grossly apparent tumor in the center. This has not been seen in the strain of origin of the tumor.

Other incidental and tentative observations on the effect of parity are as follows. The tolerance persists for at least 150 days. It does not result from mere exposure to the male in partitioned cages without mating. Apparently a previously existing immune state may be abrogated by multiparity. Finally, when females are immunized against tissues of a given strain, matings to males of that strain may result in fewer fertile matings.

We continue varied attempts to learn something of the nature of the antigen in tumor immunity and how it may differ from the associated blood group antigens. Briefly, our observations continue to show that the two antigens differ in their lability to heating, to formalin, and to sonic vibration, whether the antigenic material is derived from blood or from tumor tissue. The tumor immunity antigen is present in fetal cells before the known blood group antigens appear. The latter is first seen when hybrid embryos (i.e., containing only a half-dose) are used as antigen. When a BALB/c tumor is inoculated into C57BL/H-2^{bm} (a congenic line) recipients the latter are resistant to a DBA/2 tumor. All these materials are the same at the H-2 locus and none of the 3 possible combinations yield homograftings in unimmunized animals although tumor immunity is strong.

This problem seems at present to be getting more complicated. There are several indications that some of our ideas may be too simple and direct. It appears to be that skin grafts may permit an approach to problems for which tumor grafts are unsuitable. But we are not satisfied with present techniques for skin grafting. Accordingly we are trying to develop a technique for obtaining dependable results when many grafts are placed simultaneously on a single recipient. Much progress has been made and we believe that the objective can be reached. If this can be done it seems possible to set up experiments, involving "crisscross" and "round robin" types of transfer, that will permit observation and mathematical analysis of multifactor effects in a way not heretofore possible. Thus far, fair success has been attained with as many as 20 grafts on a single mouse. As an incidental finding, we have confirmed the reported relative incompatibility of a male graft on a female recipient within strain C57BL.

As background for some of the above it has been necessary to do a large amount of serology. We have completed determinations of blood factor antigenicity using the 7 strains under study in this laboratory. The use of each strain as both recipient and donor has yielded a 49 place table that should prove a useful reference in future work.

Dr. Maloney has reported a virus derived from our Sarcoma 37. His work was done with BALB/c mice. We have therefore autopsied a large number of strain A mice (in which our tumor is carried) for signs of leukosis and have found none. Also, Dr. Kreyberg is furnishing "tolerant" females of a resistant strain to Dr. Maloney to see if this sort of tolerance affects resistance to virus.

We have autopsied about 50 old mice of strain C57BL/6-2^{1} to see if, as suggested by Miss Uphoff, spontaneous polyps of the duodenum and colon occur. They occur in about 10% of males and about 50% of females at about 18 months of age. The two lesions tend to occur together.

A large amount of time has been spent on editorial duties with the Journal of the National Cancer Institute and Cancer Research, and occasionally for other journals.

I participated, by invitation, in the following meeting.

Francis Delafield Hospital, New York, gave a general seminar.

American Association of Head and Neck Surgeons, Washington, invited discussor.

Undisclosed Seminars, Palm Beach, a week of seminars on genetics and immunology.

Significance: The field with which this work is concerned is so difficult and obscure as to make clear definition and outline almost impossible. Nevertheless, there is a slowly growing body of evidence which indicates that there are interactions between tissues and their host which involve critical factors in the phenomena of cancer, embryology and transplantation of normal tissues. A defensible definition of cancer cannot be given today except in descriptive and biologic terms. The terms usually included things related to the experiments that we have been describing. The experiments may be significant in several ways both practical and theoretical.

The most important implication is that some of these results suggest progress toward understanding of what controls the growth of a cancer and how this differs from normal tissues. We have already seen that a tumor's power to overcome host resistance can be altered; its rate of growth, tendency to regress, and tendency to metastasize are all subject to experimental change. The host's reaction can be altered experimentally, not only between similar hosts, but also at different times and sites in the same host. The changes in the tumor have eluded morphologic, chemical, or serologic definition. One of the antigens which changes the host would be missed by current chemical methods. An important aspect of the "hybrid phenomenon" is its possible relations to "transduction." Important possibilities with regard to the effects of parity can be imagined in relation to several complications of pregnancy including H0 disease and choriocarcinoma. Interpretations are unsure. However, one might attain a biologic description of the factors that sometimes cause spontaneous tumors of man and animals to regress and that control metastasis. It has already been observed in this laboratory (and confirmed elsewhere) that such factors materially affect the outcome of many chemotherapeutic experiments.

Relation to others: There is a complex theoretical interlocking between this work and the work of Drs. Weiss, Law, Merwin and Sanford, and Miss Uphoff. Many of the results attendant upon the use of Millipore filters were foreshadowed in the work on the nature of the antigen in tumor immunity. The work on adaptation has a strong relation to recent developments in radiation biology and in tissue transplantation.

Proposed course: Extension of the main experiments described above. At present, our major need is for tumors with which experiments like the above can be repeated but with certain variations, especially a reversal of the interstrain relationships. We are studying several tumors in a search for suitable ones.

Part B. included: Yes X No

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Part 3 Honors, Awards, and Publications

Publications:

Bennett, M. K.: Some observations on the nature of the "antigen" in "tumor immunity", Acta. Un. Internat. contra cancrum 15: 548-552, 1959.

Ferguson, R. J.: Effect of inoculation of packed erythrocytes on survival of skin homografts in rats. Proc. Soc. Exp. Biol. Med. 101: 744-747, 1959.

Ferguson, R. J. and Bennett, M. K.: "Tolerance" in postpubertal female mice induced by parabiotic matings. J.Nat. Cancer Inst. (In press.)

Serial No. NCI-421
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Md.

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Individual Project Report
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Part A.

Project Title: Genetic studies in mice. A. Tumors in mice

Principal Investigator: Margaret K. Deringer

Other Investigators: W. E. Heston, M. K. Barrett

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

A. Tumors in mice.

1. Occurrence of mammary tumors in agent-free DBA/2eH sub-...

Objectives: Strain DBA/2eH, a substrain of strain DBA/2, was produced by the transfer of fertilized ova from strain DBA/2 to strain C57BL. The development of mammary tumors and of other tumors is being studied in groups of virgin, breeding, and foster-bred females, and in males treated with stilbestrol.

Methods Employed: A group of DBA/2e females was kept as virgins and another group is being foster-bred. The foster-bred females are isolated when pregnant. After the birth of the litters, the young are discarded, and the mothers are returned to the breeding cages at once. This procedure is continued throughout the breeding period of the females. Cholesterol pellets, weighing 6 to 8 mg. and containing 10% diethyl-stilbestrol, were implanted subcutaneously in the right ...

of DBA/2e males that were one to two months of age. The breeding females were those that were used to maintain the colony of DBA/2e mice. The various groups of mice are observed at regular intervals for the development of mammary tumors.

Major Findings: A considerable number of the strain DBA/2eB animals of this experiment are still alive but a sufficient number have been autopsied to indicate the probable trend of the results. The incidence of mammary tumors appears to be considerably lower in agent-free DBA/2eB animals that developed from transferred ova than in strain DBA/2. In the colony of strain DBA/2 (with the agent), 65 of 81 breeding females (80.2%) have developed mammary tumors at an average age of 14.5 months. Mammary tumors have developed in one strain DBA/2e breeding female (at 20 months of age) and in one DBA/2e male (treated with stilbestrol) at 12 months of age. No mammary tumors have been observed in any of the DBA/2e females kept as virgins (approximately one-fourth are still living but are over 19 months of age) or in any of the force-bred females (most of the animals of this group are still alive and only one-fourth are more than a year of age). These results may be contrasted with those obtained previously for strain agent-free C3HeB. Mammary tumors occurred in 4% of virgin females, in 54.6% of breeding females, in 74.4% of force-bred females, and in 22.4% of males treated with stilbestrol.

Significance to the program of the Institute: This experiment will supply information concerning the development of mammary and of other types of tumors in a strain of mice deprived of the mammary tumor agent by the procedure of transferring ova from an inbred strain of mice carrying the agent to another strain without the agent. The results will reflect on the interpretation of the relative effects of the genetic constitution, the mammary tumor agent, and the hormonal influence on the development of mammary tumors.

Proposed course of the project: Further data on the development of tumors in the force-bred females will be collected. When autopsies have been performed on the remaining virgin females and on the force-bred females, the information obtained will be combined with that from the females of the breeding colony and the material will be prepared for publication. The strain of mice will be continued and the development of mammary tumors will be studied in subsequent generations.

2. Production of tumors of the skin

Objectives: To attempt to produce tumors of the skin in strain HR mice by painting with urethan. Since it has been stated that urethan is "more-carcinogenic for skin epithelium in the mouse" it seemed worthwhile to treat mice of strain HR, which are highly susceptible to the development of tumors of the skin, with this compound.

Methods Employed: A 40% solution of urethan in ethylene glycol is painted on the skin of haired and hairless animals of both sexes of the HR strain. Other groups of males and females are painted with ethylene glycol alone. Twice weekly paintings are started when the animals are 2½ to 3½ months of age and are continued throughout the lifespan of the animals.

Major Findings: Data obtained indicate that urethan is probably not carcinogenic for the skin of strain HR mice. However, tumors of the skin have been observed in 8 of 18 hairless females and in 3 of 8 hairless males that have been painted with urethan in ethylene glycol. Two of 4 hairless males, painted with ethylene glycol alone, developed tumors of the skin. None of the tumors arose in the painted area.

Painting the skin with urethan does induce pulmonary tumors and hemorrhagic lesions of the liver in haired and hairless animals of strain HR. Some of the lesions in the liver can be classified as hemangioendotheliomas while others appear to be blood-filled spaces.

Significance to the program of the Institute: This study will add information concerning the influence of genetic constitution on the development of tumors. The effect of a carcinogen on the development of a particular type of tumor, to which the skin of animals is susceptible, will be determined.

Proposed course of the project: Groups of strain HR males and females (both haired and hairless) will be painted with urethan in ethylene glycol twice a week, and with ethylene glycol alone. The animals will be observed continuously for the development of tumors of the skin and of other lesions.

3. Influence of the lethal-yellow ($A^y a$) gene on the development of leukemia in mice.

Objectives: To determine the relationship of the lethal-yellow gene (known to increase body size) and the occurrence of leukemia in treated and untreated animals (in cooperation with Dr. Heston).

Methods Employed: Crosses have been made between strain DBA/2, a strain susceptible to the development of leukemia, and strain IRR, a strain carrying the lethal-yellow gene. The F_1 mice have been painted 3 times a week for 7 weeks (7 different painting sites have been used) with 0.5% 3-methylcholanthrene in ether. A second group of F_1 animals has been maintained without treatment. The two groups of F_2 are observed at regular intervals for the development of leukemia.

Major Findings: The large majority of the treated and of the untreated animals of this experiment are still alive. No reticular neoplasms of any type have been observed in any of the animals that have been autopsied.

Mammary tumors have developed in 13 of the untreated females and in 1 of the treated females. All of these females were the yellow segregants.

Significance to the program of the Institute: This experiment will demonstrate the role of the lethal-yellow gene in the development of leukemia in mice.

Proposed course of the project: The group of untreated animals and the group painted with 3-methylcholanthrene will be observed at regular intervals for the development of tumors and of other lesions.

4. Necrotising arteritis in strain BL/De mice.

Objectives: During routine autopsies of breeding female mice of strain BL/De, the ovaries were noted to be enlarged and pale. This suggested ovarian tumors and therefore histologic sections of these ovaries were made and examined. The microscopic examination explained the gross appearance for it was found that the corpora lutea were heavily infiltrated with amyloid and enlarged. This finding was of particular interest, because this same strain of mice was reported by Sokoloff and Wolbergmann to have an idiopathic necrosis of bone. At the re quest of Dr. Leon Sokoloff of NIAMD a study of the available histologic material from routine autopsies of a group of breeding females was made and the material was presented, by invitation, at the Conference on the Comparative Pathology of Arthritis and Rheumatism held at NIH on February 5 and 6, 1959.

Methods Employed: A study was made of the histologic sections of the ovaries from a group of breeding females of strain BL. In addition, sections of kidneys and of tumors, when present, were also available for study.

Major Findings: On gross examination the ovaries of strain BL were often observed to be enlarged and pale. On microscopic examination the sections revealed infiltration of amyloid into the corpora lutea. The extent of infiltration varied from small foci to large rounded areas entirely replacing the corpora lutea. Sometimes there was calcification in the central portion of the amyloid mass. Amyloid was observed in one or both ovaries of 59 of the 73 breeding females of strain BL. In addition to amyloid, striking vascular lesions were observed in one or both ovaries of 32 breeding females. The vascular lesion has been described as a thickening of the walls of the vessels due to the deposition of eosinophilic material within the intima and the media. Pyknosis and fragmentation of the nuclei occurred in such areas. The presence of arteritis was not detected on gross examination either in the ovaries or in other organs. Therefore, no data on the distribution of the lesion are available since complete microscopic surveys were not done. However, the vascular lesion was noted in microscopic sections from the perirenal adipose tissue, in vessels of the adrenal cortex, in vessels of the uterus, and in vessels of the mesentery. In addition to the vascular lesion, renal disease was observed in 65 of 73 breeding females. Microscopically, the renal disease was the type that has been described by Dunn and that occurs particularly in strain A mice. Mammary tumors occurred in 12 of the 73 breeding females between the ages of 11 and 22 months and pulmonary tumors in 16 of 73 females between 15 and 23 months.

Significance to the program of the Institute: This study has provided more information concerning the characteristics of a highly inbred strain of mice. This will increase their usefulness in the study of various diseases.

Proposed course of the project: The material has been prepared for publication in the Proceedings of the Conference on the Comparative Pathology of Arthritis and Rheumatism which will be published in the November issue of Laboratory Investigation.

5. Amyloid in the organs of strain BL mice.

Objectives: The observation that the corpora lutea of the ovaries of strain BL breeding females were often heavily infiltrated with amyloid prompted the present study of the distribution of amyloid in the organs of strain BL mice.

Methods Employed: Groups of male and female mice of strain BL have been set aside and will be autopsied at 15 months of age. Sections of all organs will be made and data on the distribution of amyloid will be secured from them.

Major Findings: No animals have been autopsied and therefore no results are available.

Significance to the program of the Institute: This study will provide more information concerning the characteristics of one of the highly inbred strains maintained at the Institute.

Proposed course of the project: Data on the distribution of amyloid will be secured and the material prepared for publication.

6. Immunogenetic experiments.

Objectives: To produce special types of animals for collaborative experiments with Dr. M. K. Barnett (see Dr. Barnett's report for details).

Part B included Yes X No

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Deringer, M. K.: Mammary tumors and hepatomas in agent-free strain C3HeB mice. Acta Unio. internat. contra cancr. 15: 150-151, 1959.

Deringer, M. K.: Occurrence of tumors, particularly mammary tumors, in agent-free strain C3HeB mice. J. Nat. Cancer Inst. 22: 995-1002, 1959.

Deringer, M. K.: Necrotizing arteritis in strain NL/Be mice. Laboratory Investigation (November 1959).

Heston, W. E., Vlahakis, G., and Deringer, M. K.: High incidence of spontaneous hepatomas and the increase of this incidence with urethan in C3H, C3Hf, and C3Be male mice. J. Nat. Cancer Inst. 24: (Feb. 1960).

Heston, W. E., Vlahakis, G., and Deringer, M. K.: Delayed effect of genetic segregation on the transmission of the mammary tumor agent in mice. J. Nat. Cancer Inst. 24: (Mar. 1960).

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Serial No. NCI 427
1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

[redacted]

Studies on the etiology and therapy of experimental lymphomas

L. W. Law

Man years (calendar year 1959)
Total: 4 2/3
Professional: L. W. Law, 2/3
Other: 4

[redacted]

1. The experiments are now nearly completed relating to the influence of thymic grafts (C57BL) introduced into millipore chambers, intraperitoneally, in thymectomized, fractionally irradiated C57BL and BAF$_1$ hybrids. Despite the persistence of tissue for long periods of time within the chamber, no evidence of transformation to neoplasia has been seen. Approximately 20-30% reticular neoplasms, type A and B of Dunn have been observed but these are unrelated to thymic tissue. Interestingly, no plasma cell neoplasms have been observed in mice of these genotypes although BALB/c and C3H mice appear to be sensitive to the plastic material.

2. Bone-marrow chimeras, developed in the C3Hf/$_{Lw}$ and C3Hf/$_{Bi}$ strains of mice by introduction of AKR bone marrow into <12-hour old mice have developed lymphocytic neoplasms at a frequency and

Part B included Yes

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Part A.

latent period of non-chimeric controls, nor has the introduction of thymic AKR grafts into such chimeras resulted in an influence of the leukemic picture. Approximately 10% of the Bittner subline recipients have presented unilateral parotid tumors when receiving bone marrow from AKR donors known to be HI positive.

3. Isologous bone marrow given immediately following the last of 4-weekly irradiations (acute X-ray) completely prevented the appearance of thymomas in thymic grafts introduced at 3, 7, 14 and 21 days after irradiation on C57BL and BAF_1 mice. Thus, the inhibitory effects of isologous bone marrow is a permanent long-lasting influence. As discussed above the presence or absence of thymic tissue, and as shown here, the introduction of isologous bone marrow are factors which do not influence other neoplasms of reticular tissue. Such neoplasms, chiefly involving monocytes, appear after 12-15 months of age in these mice.

B. X-radiation and lymphomas of mice

1. A study is in progress of the effect of total body X-radiation on the induction of lymphomas in C57BL/Ka and RFM mice, irradiated in utero at 10.5 days and at later periods just prior to p[illegible] at 17 to 19 days. The 10.5 day schedule was se[illegible] as a result of the findings of Russell and Major [illegible] the induction of somatic cell gene mutations i[illegible]

Part I.

characters in mice irradiated at a presumably very sensitive age, 10.5 days. To date many of the groups have passed six months of age and no evidence has been observed of an increased frequency, nor of earlier appearance. A negative outcome in these studies could conceivably rule out any striking sensitivity of fetal tissues but could not, because of numbers of animals alone, rule out a probable influence comparable to what may occur in human beings to the possible response of the fetus to radiation received during roentgenographic examination of the mother.

2. Work is continuing on the comparative sensitivities to fractionated whole-body irradiation of 3 sublines of C3H mice, C3Hf/$_{LW}$, C3Hf/$_{Bi}$ and C3H/$_{Fg}$, the latter a high-leukemic line studied in this laboratory over the past several years. Several interesting observations are worth considering:

a. C3Hf/$_{LW}$ mice are relatively resistant. To date only 20% lymphocytic neoplasms have been recorded.

b. Samples of C3Hf/$_{Bi}$ mice differ in their responses. Our subline C3Hf/$_{BiLW}$, maintained for several years here develop 50-60% thymic lymphomas. A sample of these obtained directly from Bittner were not more sensitive, however, than the C3Hf/$_{LW}$ subline.

c. An early appearance of thymic lymphomas is [illegible] appear in this subline and thymectomy does not influence the spontaneous occurrence of the reticular neoplasms. In this connection a collaborative study

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has been undertaken with Dr. John Maloney in an attempt to recover a cell-free material from C3Hf/Fg spontaneous neoplasms using the Maloney technique. Heretofore, extracts and filtrates and tissue culture materials have been ineffective in <12 hour old test mice of various strains.

3. A study in collaboration with Drs. Rowe and Hartley, NIAID, has been concluded concerning the relation of infection of parotid tumor agent (polyoma) to lymphocytic neoplasms in AKR, C58 and C3Hf/Bi (X-ray induced) mice and the observations submitted for publication. There was found to be no correlation of HI antibody with leukemia appearing at various times throughout life, nor was there obtained any evidence to indicate a greater risk of developing leukemia in mice infected with polyoma virus.

4. As soon as facilities are available to this section, it is planned to initiate a study using chronic gamma irradiation; of the influence of dose rate on the induction of reticular neoplasms in C3HEL/Fg and DBA/2 mice. Dosage intensities approximately 5 r to 80 r/hour will be used in which the same total dose is given over the same [illegible] time. This will be accomplished in one strain for intact and for thymectomized mice in order to differentiate the influence on thymic and non-thymic neoplasms

Item 6.

C. Cell-free and transplantable viruses and the induction of leukemia and other neoplasms.

Various phases of our work are done on a collaborative basis with Drs. Rowe and Hartley, L. Dr. Dawe, L. Path., and Drs. Kahler and Lloyd, L. Physiol., NCI.

1. Preliminary results have been reported of the relationship of antibody status (HI) of the mother of several strains and sensitivity of the litters to the carcinogenic stiumlus of a P-388 passaged highly virulent parotid tumor virus. All mothers showing HI antibody titers >200 delivered young which resisted (tumor induction) virus. Spontaneous convertors', tumorous mothers or those infected by suckling their inoculated litters all behaved similarly. An additional resistance factor, not related to antibody, needs further study. This is reflected in absence of tumors in nearly 30% of mice, randomly spread throughout litters. Another interesting observation concerns the appearance of a significantly greater number of mammary tumors, over controls in $(C3H \times AKR)_{F1}$ mice beyond 8 months of age; these mice completely resisted the early carcinogenic effects of virus. The possible relationship of this phenomenon to antibody titers needs investigation.

Foster nursing of litters borne to HI positive mothers on HI negative mothers and vice ver HI antibodies to be transferred to litters t transplacentally and through the mother's milk. Transfer by either route appears equally efficac

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2. A study of the comparative effect on CPE, hemagglutination titers, CBM and infectivity (tumor induction in mice) in relation to number of virus particles is underway. Several interesting (apparent variants) are being investigated: a) a thymotropic substrain, b) a strain giving rapid CPE but with an unusual induction of tumor types in mice, c) a strain with high HA titer, giving rapid CPE which induces neoplasms in hamsters but apparently not in mice, and d) a hamster kidney-passaged strain which is non-carcinogenic for mice. Titrations down to 1 x 10^{-7} (particle count <30) are being studied.

3. Other projects being continued are:

Sensitivity of HI-negative adult C3Hf/Bi and [illegible] the role of thymectomy in response to thymotropic virus.

Transformation in in vitro organ cultures of salivary glands to parotid tumor agent. A preliminary report is in press. This work is to be continued by Dr. Clyde Dawe, L. Path., NCI.

Study of the host cell-virus relationship in a "carrier line" of P-388 in which complete CPE does not occur.

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Part A.

D. Studies of mechanisms involved in inhibition of leukemic cell growth

A collaborative study has been initiated with Dr. Wallace Brockman, Southern Research Institute investigating the biochemical mechanisms of resistance to 5-fluoropyrimidines. The sublines available for study developed in this laboratory are: $P815/_{FU}$, $P815/_{FUR}$, and $L1210/_{FO}$. The biological characterization and standardization of these lines have been completed. At present the biochemical work involves: 1) incorporation studies in sensitive and resistant sublines of orotic vs. fluoorotic and uracil vs. fluorouracil 2) capacity of S and R sublines to metabolize ribonucleotide and derivatives. It is planned to study the enzyme changes, particularly kinase and phosphorylase in all variant lines since Heidelberger et al. (Nature 181, 939, 1959), have shown differences for both enzymes but involving different neoplasms, during the stepwise development of resistance. Uracil instead of 5-fluorouracil, however, was used.

A study has been initiated testing for probable collateral sensitivities to Amethopterin, 6-mercaptopurine, cytoxan and DON of several variant lines of the mast-cell neoplasm P815. This has been prompted by the observation that certain L1210 variant lines, particularly $L1210/_{TG}$ and $L1210/_{6MP}$, show a strikingly increased sensitivity to the 5-fluorinated pyrimidines.

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Part A.

E. Studies in mammalian somatic genetics and carcinogenesis

Within the next year it is proposed to establish a new experimental method to study certain basic problems concerning carcinogenesis and mammalian somatic cell genetics in collaboration with Drs. Rott and Bradley, University of Melbourne. Guest Investigators at NIH. The method a "continuous injection technique" provides that tissues either in situ or grafted can be continuously, directly and locally influenced by the infusion of materials.

Attempts at genetic transformation of cells will concern:

1) Alteration of histocompatibility characters of tumor tissue using coisogenic strains of mice.

2) Alteration of drug sensitivity to drug resistance using established variant lines.

One experimental situation pertaining to mechanisms of carcinogenesis that will be studied concerns the possibility of influencing thymic lymphoma induction in C57BL mice. Here thymic grafts will be made into the renal capsule of fractionally irradiated mice and attempts made by the use of different substrates (NA) to influence lymphoma production and regeneration of thymic graft.

3) Attempts to transform normal cells (salivary) glands (for example), to neoplasms through continuous infusions of DNA-extracted malignancies.

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Part A.

Dr. Roosa, recipient of an NIH Postdoctoral fellowship, is continuing his tissue culture studies.

1. Nutritional requirements of P-388 in cell culture

As noted in last year's report an established cell culture was found to grow optimally in Eagle's Basal Media supplemented with only 5% whole calf serum. However, when the serum was dialyzed growth did not continue. For sustained growth in mass culture using dialyzed calf serum it was found that the addition of pyruvate or L-serine restored the growth promoting activities of the whole serum. Subsequent work showed that for isolated (single) cells both pyruvate and L-serine (or glycine) were necessary for growth.

By the use of radioactive tracer experiments we attempted to determine the metabolic relationships between these compounds and P-388 lymphoblasts. The cells were allowed to grow in the dialyzed serum medium in the presence of either radioactive glucose, pyruvate or serine and after a 10-fold increase in protein had occurred the specific activities of the protein-serine, glycine and alanine and the pyruvate in the medium were determined. These experiments showed that all of these compounds were synthesized from glucose. It was also established that L-serine, glycine and alanine in the cellular protein do not arise from exogenous pyruvate, and that the effective supplements therefore act in some way other than as simple precursors for these amino acids.

[illegible]

3. [illegible]
4. (Leukemia Studies [illegible]
5. (Bethesda 14, [illegible]

[illegible]

[illegible] establish that pyruvate can be [illegible] by [illegible] both the [illegible] to [illegible] and [illegible] cells. [illegible] late and α-keto-n-caproate serve as growth factors in mass culture only. A series of other keto acids, fatty acids, Kreb's Cycle intermediates, purines, pyrimidines, vitamins and amino acids were found to be ineffective.

2. Development of methods of analyzing the [illegible] characteristics of mouse cells [illegible] in vitro and in vivo techniques.

With the development of an adequately defined medium it became possible to grow populations of P-388 cells both in mass culture and from single cells. Studies with large populations were usually observed by the use of protein determinations and these studies were often supplemented with nuclei counts. By allowing a few cells to develop in a petri dish maintained in an incubator having a constant CO_2 atmosphere we are able to isolate clonal stocks. We are then able to study these clones quite readily as they develop into colonies under the pressures of various growth inhibitors.

The intraperitoneal injection of these cells [illegible] an ascitic tumor. One can then test the genome of the resultant tumor either by in vivo treatment or by plating small numbers of cells in tissue culture and counting the resultant colonies in the presence of various concentrations of inhibitors.

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Part A.

3 Selection of drug resistant variants

Variant lines of P-388 cells were obtained by selection of resistant sublines growing in the presence of gradually increasing concentrations of various growth inhibitors. Several distinct lines have been obtained and are carried continuously in different concentrations of these drugs. To date distinct resistant steps to Amethopterin (representing a final 100-fold increase over the minimum inhibitory level) have been selected. The isolation of these variant lines has followed a stepwise pattern and each step has paralleled a similar step in cross-resistance to Aminopterin. Other resistant lines include two steps to four other inhibitors; 5-Fluorouracil, 5-Fluorouridine, 5-Fluorodeoxyuridine, and 8-Azaquanine. In addition to these lines several variants containing double markers have been isolated. Due to the recent isolation of these lines only one has been tested. Under cloning conditions this line retains a well marked resistance to both 5-Fluorouridine and Amethopterin. The other double marked lines are resistance to 5-Fluorouracil and Amethopterin, and 8-Azaguanine-5-Fluorouridine resistance, and an 8-Azaguanine-Amethopterin resistance.

The resistance obtained in each case is heritable as it is retained during growth in the absence of any inhibitor for periods of greater than 100 generations.

Part I.

It is possible to obtain ascitic tumors after the intraperitoneal injections of from 1-3 million cultured cells in histogenitically compatible mice. We are interested in determining if the resistance obtained in culture is retained after animal passage. Results here are somewhat slow due to a lengthy latent period, but initial results show a retention of resistance in mice although it is still of a low level.

Experiments are in progress at the present time to obtain resistant populations of these cells by *in vivo* selection. These lines when obtained shall also be tested in cloning type experiments to determine the level of resistance and the percentage of isolated cells capable of *in vitro* growth in the presence of various concentrations of inhibitors.

4. Preservation of variant cell lines

It has become advisable to preserve the many variant cell lines being established. Thus they would be available if a culture line became accidentally contaminated or if further work with a particular resistant step became warranted at some later date.

We chose to explore the possibilities of [illegible] technique using apparatus already available. To date all but one of the resistant steps have been successfully preserved. The longest test period has been a period of six months.

[illegible]

[illegible]

1. [illegible] cells in cell culture

[illegible] studies with Dr. Michael Potter of [illegible] Laboratory, include attempts at growing plasma cell tumors in cell culture. These studies are aimed at developing a relatively simple system for establishing clones of [illegible] cells so that the [illegible] patterns of tumors arising from single cells may be studied. [illegible] could be used to explore [illegible] growth. It is anticipated that through these methods it will be possible to determine if the Bence Jones protein and the serum protein are produced by the same or different cells.

2. [illegible] produces a cytopathic effect in P-388 cells

Studies are in progress using a parotid tumor agent (PTA) obtained from a normal tissue source (AKR bone marrow). This agent apparently replicates in P-388 lymphoblasts in a media containing only calf serum. To the present time fourteen successive passage generations of this agent have been completed. When the wash fluids at the time of culture lysis are injected into newborn mice a variety of tumors (predominately of the parotid gland) appear after six weeks.

[illegible] mouse strains which have currently developed this agent include C3HF/Bi and (C3H x AKR)F$_1$'s. There does not appear to be any weakening of the oncogenic properties of this agent after continuous tissue culture passage, nor is there a significant change in the type of tumor arising after continuous passage in cel[illegible]

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Part I...

Experiments are already in progress to determine if the activity of the sample is altered by alternating passages in cell culture and animals. The reisolation of active material has been successfully accomplished from a [illegible] tid tumor in the first attempt and unsuccessful when attempted from a mammary tumor.

Collaborative experiments already in progress with Doctors Law , Dawe, Rowe, and Kahler are concerned with studies of several variants of this agent. Included are examinations for cyto-pathogenicity of tissue culture lines, mouse sensitivity and hemagglutination.

7. Organ culture studies

Studies are currently being planned with Dr. Law to study the effects of the Maloney Agent (and possibly other viral-like agents) on thymic and other tissues in in vitro organ type cultures.

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Part B.

Honors, Awards, and Publications

Dawe, C. J., Law, L. W., and Dunn, T. B.: Studies of parotid tumor agent in cultures of leukemic tissues of mice. JNCI, 23: 717-797, 1959.

Law, L. W., Some aspects of the etiology of leukemia. Proc. of the Third Canadian Cancer Conference, pp. 145-169. Academic Press, N.Y. 1959.

Rowe, W. P., Hartley, J. W., Law, L. W., and Huebner, R.J., Studies of mouse polyoma virus injection III. Distribution of antibodies in lab. mouse colonies. J. Exp. Med. 109: 449-462, 1959.

Rowe, W. P., Hartley, J. W., Brodsky, I., Huebner, R. J., and Law, L. W. Observations on the spread of mouse polyoma virus infection. Nature, 182, 1617, 1959.

Uphoff, D. E., and Law, L. W. An evaluation of some genetic factors influencing irradiation protection by bone marrow. JNCI, 22: 229-241, 1959.

Dawe, C. J., and Law, L. W., Morphologic changes in salivary-gland tissue of the newborn mouse exposed to parotid-tumor agent in vitro. JNCI, 23: 1152-1178, 1959.

Law, L. W., Dawe, C. J., Rowe, W. P., and Hartley, J. W. Antibody status of mice and response of their litters to parotid tumor agent (polyoma virus) Nature, 184: p. [illegible], 1959.

Chosen a delegate (USA) to the WHO Expert Advisory Committee on Cancer.

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Part A.

Studies of the etiology, pathogenesis and chemotherapy of reticular neoplasms in mice

Michael Potter

Man years (calendar year 1959)
Total: 4
Professional: Potter, 1
Other: 3

A. Studies on drug resistance

Two questions on drug resistance have been asked:

1) When do resistant cells develop?

2) What component of the cell changes when a sensitive cell becomes resistant? A gene? Employing one model system the DON-sensitive ascitic mast cell neoplasm P815, evidence was obtained that resistant cells could develop in the presence of the antagonist. However, the demonstration of persisting sensitive P815 cells in the face of long courses of DON was a disappointing development. A drug sensitive tumor which would be destroyed by a drug is essential to the purpose of demonstrating when and how resistance develops.

Three model systems are being explored for their value in contributing more information.

Part B included Yes

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Part A.

1 a. An Amethopterin sensitive, ascitic, tetraploid lymphocytic neoplasm: P288

Like other DBA/2 lymphocytic neoplasms, greatly prolonged survival times are obtained with Amethopterin treatment. Full resistance to amethopterin does not develop in a single transfer generation, yet Amethopterin fails to cure mice inoculated with doses of 10^5 or more P288 cells. A technique has been developed to explore the question. The inoculation of 10 cells will initiate a successful transplant in about 80% of mice. Five treatments of Amethopterin 3 mgm/kg given on days 0,2,5,7,9, to mice inoculated with 10 sensitive cells results in the development of a transplant in less than 5%; with 10 cells of a full resistant line about 80%. Cells were isolated from mice inoculated with sensitive cells after Amethopterin treatment of 25-30 days duration. When doses of 10 of these cells were inoculated into mice, the above-mentioned Amethopterin treatment resulted in successful growth in roughly 30-60% of the cases depending on the isolate. Mice following Amethopterin treatment which developed ascites did so at a much later time than similar mice inoculated with a full resistant line, suggesting the cells were not growing during a treatment but were persisting. Evidence has been obtained that this characteristic is heritable. The finding explains that sensitive cells can change to a resistance though this is not a complete resistance.

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1 b. With Dr. George Briggs, a folic acid deficient diet is currently being studied for its effect on P288 and P288 AmR2. Surprisingly the results now nearing completion, consistently demonstrate the folic acid deficient diet produces a better survival time effect in mice bearing the Amethopterin resistant subline than the Amethopterin sensitive subline.

2. The search for a supply of cortisone sensitive lymphocytic neoplasms

Cortisone sensitivity in lymphocytic neoplasms of mice mimics in many ways the response of human acute leukemia to cortisone, i.e., the regression of large established transplants, followed by recurrence, is similar to the profound short-lived remissions in man. One cortisone sensitive neoplasm (P1798) failed to maintain its sensitivity over an extensive period. A search for new neoplasms is in progress. Several methylcholanthrene induced lymphocytic neoplasms of DBA f/2 origin have failed to respond. X-radiation induced thymic leukemias of strain C57BL are being tested. A good working model system of this type should throw light on the time of origin of cortisone resistance in relation to the initiation of steroid treatment.

3. 5-Fluorouracil sensitive plasma-cell neoplasm of strain BALB/C origin. MPC 1.2.4

Established transplants of 2 cm. in diameter can be completely regressed with 5-fluorouracil. A fourth MPC plasma-cell tumor, MPC-3 fails to respond to 5-fluorouracil. This model system has great potentiality; but is in need of intensive exploration.

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Doses of 5-fluorouracil (20 mgm. kg.) given daily (6 times weekly) are the most effective. Toxicity results after 2-3 weeks and the dose must be changed to 10 mgm. kg. This maintenance dose can be given over a 2-month period but the tumors recur in the presence of this treatment. The development of 5-fluorouracil resistance is in progress. MPC 1,2 are currently in ascitic form. The ascites are useful for initiating the tumor with controlled doses of cells. The ascitic tumor cell population, however, is sparse. Mice with fully regressed MPC-4 of several months duration accepted a new graft of MPC-4 and died. The opportunity to explore immunity is presented. The development of this system in a quantitative way should provide an excellent means for learning more about when and how resistance in cells arises.

B. Experimental Multiple Myeloma

1. Induction of plasma-cell neoplasms in mice

A controlled means for producing plasma-cell neoplasms in mice would provide a methodology to learn certain facts regarding multiple myeloma.

A new means for producing plasma-cell neoplasms is under investigation. Following the observation of Dr. Ruth Merwin that millipore diffusion chambers implanted intraperitoneally in mice cause the appearance of plasma-cell neoplasms in BALB/C mice, other means have been sought. Sixty-four mice were given incomplete Freunds

Part A.

adjuvants and a filtrate of a culture of staphylococcus intraperitoneally. One-half were painted with methylcholanthrene; the other were controls. Two plasma-cell neoplasms have appeared in the MCA group and two in the controls. These resemble grossly and microscopically the tumors described by Merwin.

Mice immunized intraperitoneally with horse serum before the adjuvant injection are being similarly observed. It is hoped that by stimulating specific antibody formation before or during carcinogenesis, that a myeloma globulin producing transplantable plasma-cell neoplasm can be obtained which will permit a study of the antibody activity of the myeloma globulin.

2. Investigation into the nature of myeloma globulins

Currently 10 transplantable plasma-cell neoplasms are being maintained in this laboratory. Employing the technique of immunoelectrophoresis, the myeloma globulins are being characterized. The myeloma globulins produced in mice bearing MPC 2 and 3, of Beta mobility on paper, have been found by immunoelectrophoretic analysis to be antigenically unrelated to a gamma 2 globulin or any other globulin in normal serum.

These two identical myeloma globulins can be clearly and strongly precipitated in agar by rabbit anti MPC-3 serum, or by rabbit anti MPC-3 serum, or by rabbit anti MPC-3 serum absorbed with whole normal BALB/C serum, but are not developed by rabbit anti-normal BALB/C serum. The list of tumors in BALB/C is being extended, 6 are now available to determine whether there is an infinite or grouped antigenic variation.

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C. Studies in methylcholanthrene (MCA) induced in strain DBA f/2 Lw

1. Mice of strain DBA f/2 Lw develop generalized leukemia very rapidly following MCA painting. Mice of one month of age are begun on a course of 20 paintings over a period of 7 weeks. Four weeks following the last painting the first wave of leukemias appear. Nine cell free filtrates were prepared from leukemia tissue of individual cases arising early and from pools of several donors and inoculated into newborn mice of strain DBA f/2. Leukemia tissues were ground in sand and 10-20% extracts were centrifuged lightly and passed through Selas filters. To date in 159 mice under observation, for a minimal period of 5 months and a maximum of 11 months no cases of leukemia have been observed.

2. A control pedigreed colony of DBA f/2 mice is now 1 year of age (minimum). The incidence of leukemia and allied disorders is being determined. This is a tedious task for old mice of the strain often die unexpectedly without forewarning.

D. Genetic Study

Mice of strain BALB/C have not developed leukemia following MCA skin painting. A study has been initiated to determine if the difference between BALB/C and DBA f/2 in response to MCA is controlled by a single, two or more genes. Mice from a pedigreed colony of BALB/C/An maintained in the laboratory have been mated with DBA; the F_1 hybrids, first generation and second generation backcross to DBA f/2 are being painted with MCA to determine the incidence of leukemia. Thus far no cases of leukemia have appeared in F_1 hybrids.

1. (Laboratory of Biology)
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Part B.

Honors, Awards, and Publications

Potter, M. Biologic studies on the development of DON resistance in a mast-cell neoplasm of the mouse. JNCI, V23, 163-181, 1959.

Potter, M., and Haas, V. H. Relationships between lymphocytic choriomeningitis virus amethopterin and an amethopterin-resistant lymphocytic neoplasm in mice. JNCI, V22, 801-809, 1959.

Fahey, J. L., and Potter, M. Bence-Jones proteinuria associated with a transplantable mouse plasma-cell neoplasm. Nature, 184,654-655, 1959.

PHS-NIH

Individual Project Report
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3. (Bethesda 14, Md.)

Part A.

Studies on Biochemical mechanisms of sensitivity and resistance to antimetabolites

Elizabeth P. Anderson

Man years (calendar year 1959)
Total: 2
Professional: 1
Other: 1

A. Studies on azaserine and diazo-oxo-norleucine.

This is a continuation of work reported in previous years on biochemical differences in the metabolism of azaserine-sensitive and-resistant lines of plasma cell neoplasm 70429.

1. Two new azaserine-resistant lines of 70429 have been developed by procedures previously used in these laboratories for the selection of drug-resistant variants. These lines are now being studied biologically, and will also be studied biochemically, in comparison with the AzR-1a line previously isolated by Dr. Michael Potter of this section and already studied in some detail by us.

Part B included Yes

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Part A.

2. Enzymatic studies on the azaserine-inhibited conversion of formyl-glycinamide ribotide to formyl-glycinamidine ribotide in 704298 and 70429 AzR-1a have been continued in the attempt to define further the quantitative differences observed between the two lines in this conversion. Toward this end some further work has been done on purification of the enzyme. Considerable work has also been carried out on the development of methods for enzymatic synthesis and for isolation of certain substrates for this work and on the accumulation of a store of these substrates.

3. Arrangements have been made for further studies on possible differences between these sensitive and resistant lines in concentrative cell transport and/or breakdown of azaserine and MON; these follow up earlier observations on differences in inhibition observed with intact cell suspensions but not with cell-free extracts. These studies will be carried out in collaboration with Dr. John Jacquez at Sloan-Kettering Institute, in the months of February and March.

B. Studies on the metabolism of ribonucleic acid

1. Work has been completed on the partial purification and the study of properties of a phosphodiesterase from ascitic leukemia L 4946. This enzyme is capable of hydrolyzing certain 5'-phosphate-ended ribopolynucleotides to mononucleoside-5'-phosphates; the mechanism of the reaction has been explored and found to involve a

Part A.

stepwise cleavage of one nucleotide unit at a time from the end of the polymer chain. Substrate specificity has been investigated; the enzyme also shows activity against both ribo- and deoxyribo-oligonucleotides, but it is unable to hydrolyze simple esters of nucleoside-5' - phosphates because of a requirement for a nucleoside moiety on each side of the susceptible phosphodiester bond. This is the first enzyme to be reported with such a substrate specificity and represents, therefore, a new type of phosphodiesterase. Similar hydrolytic activity against ribopolynucleotides has been found in all of a number of other types of tumor tissue examined. This work has been carried out in collaboration with Dr. Leon Heppel, NIAMD. A report of the work was presented at the Federation Meetings in April, a paper on the complete work has been written and will be submitted shortly for publication in Biochim. et Biophys. Acta.

C.

1. Much time in the last few months has been devoted to compilation of the literature and preparation of the text for the chapter on Biochemistry of Cancer for the 1960 Annual Reviews of Biochemistry which should be completed shortly. This chapter will be written in collaboration with Dr. Lloyd Law of this section.

D.

1. As in previous years, some time was spent in the preparation of manuscripts on the results of earlier research projects.

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Part B.

Honors, Awards and Publications

Anderson, E. P., Maxwell, E. S., and Burton, R. M., Enzymatic syntheses of C^{14}-labeled uridine diphosphoglucose, galactose-1-phosphate, and uridine diphosphogalactose. J. Am. Chem. Soc., 81: 0000 (1959)

Yen, C. Y., Anderson, E. P., and Smith, P. K., Effect of X-irradiation on incorporation of ureidosuccinic acid-C^{14} into nucleic acid pyrimidines. Radiation Res.,

Honors

Election to membership in the Society for Experimental Biology and Medicine.

Serial No. NCI-427 (C)

1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

Factors effecting transplantation of hematopoietic tissue in the irradiated mouse

Delta E. Uphoff

Man Years (calendar year 1959)
Total: 1
Professional: 1/2
Other: 1/2

In past reports, emphasis was placed upon the genetic factors influencing irradiation protection by bone marrow. From the survey which was initiated in an attempt to ascertain the role of certain genetically determined histocompatibility genes in the case of the marrow graft in the irradiated host several generalizations can be drawn. There is presumptive evidence indicating that if genetically incompatible marrow is used as a protective agent, a graft versus host reaction ('secondary disease') will occur resulting in the death of many of the treated mice. The severity of the reaction and degree of mortality varies with the strain combinations and is probably a function of the antigenic incompatibility of the strains employed. Foreign marrow has not been demonstrated to produce 'secondary disease' if marrow donor and irradiated host share the same histocompatibility-2 (H-2) phenotype. The H-1 and H-3 loci have little or no effect upon this reaction.

Part B included Yes

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Part A.

As a result of this study on the genetics of marrow transplantation, it became apparent that there are other factors influencing the long term survival of these lethally irradiated and protected mice. The premature clinical application of marrow transplantation as a therapeutic measure in terminal cases and the resulting failure to obtain evidence that the marrow transplantation was successful, emphasizes the urgent need for a more complete study of factors influencing the acceptance and survival of the marrow graft in the irradiated host. The absence of technical assistance has made progress very slow and has necessitated the abandonment or indefinite postponement of several aspects of this study.

A. Adult marrow transplantation

1. The survey of the genetic factors influencing irradiation protection by bone marrow was continued in an attempt to establish an all inclusive working hypothesis. All strain combinations tested within the H-2^b or H-2^d groups have been found to be compatible as either marrow donor or irradiated host. This lack of strain specificity but dependence upon only H-2 specificity, sets marrow transplantation apart from transplantation of other tissues. No combination was found in which good long-term survival was obtained when strains were of different H-2 phenotypes. The H-2^k group of which five inbred strains are available is more complex. The reports of hemagglutination studies on these strains indicate an incomplete matching of the antigenic components associated with the H-2 locus. In transplanting marrow in irradiated host combination of H-2^k, this incomplete matching

Part A.

of antigenic components manifests itself in a moderately severe reaction from which an appreciable number of animals survive. The presence of this 'secondary disease coupled with the serological evidence of an antigenic difference between strains of the H-2^k group would indicate that these data on the H-2^k combinations are not at variance with that of the H-2^b and H-2^d groups.

2. The new coisogenic line made available by Dr. George Snell of Roscoe B. Jackson Memorial Laboratory made possible the confirmation of the previous report that a single gene difference at the H-1 locus does not produce a visible reaction. This new coisogenic line completed a coisogenic trio and made possible the testing of the additive effect of a gene difference at the H-1 and H-3 loci simultaneously. The double gene difference was not a sufficient stimulus to produce a visible reaction.

3. In treated mice of some genetic combinations, there appears to develop a transient anemia following initial protection. In some cases this anemia appears to be related to a tendency for hemmorrhage. It was not considered an urgent problem for study since it only infrequently results in death of the host. Although it may prove profitable and enlightening, the study of this phenomena was indefinitely postponed. Anemia of a more severe nature will be discussed in a following paragraph (B 2).

Part A.

4. Homologous marrow inoculation following irradiation at the mid-lethal dosage level has been observed to be lethal within 30 days post irradiation in several laboratories. Although the genetic combinations were few (2) and the groups were small, the confirmation of these results by three different investigators led to the immediate acceptance of this phenomenon. In an attempt to determine the role of the H-2 locus at this dosage level (500r-550r), one strain of the H-2^d phenotype was selected as irradiated host, two different H-2^d marrow donors were selected as well as one each of the H-2^b and H-2^k phenotypes. At least 90% of the animals in all four groups at the 500r and 550r dosage levels survived thirty days or longer. These results would indicate that the mid-lethal zone effect may not be a problem for all genetic combinations. A limited number of strain combinations will be tested in an attempt to determine what factors are involved in this lethal effect. The mid-lethal zone effect has been considered a major problem in clinical therapy where the lethal range has not been established. The full significance of the present observation is not immediately apparent and speculation at this time would be premature.

B. Fetal hematopoietic tissue

1. In an attempt to preclude the graft-versus-host reaction fetal hematopoietic tissue was used as a protective agent. It has been reported previously that in one genetic combination no visible reaction occurred when young (14 day gestation) fetal tissue was used rather than adult tissue. Comparative data are now available on four irradiated F_1 hybrids receiving parental-strain fetal hematopoietic tissue

Part A

in seven different combinations. In several, but not all, hybrid-parental-strain combinations, no reaction was observed if early fetal tissue was used. In the other combination and with late neonatal tissue, a syndrome occurs which is less severe and more animals survive the critical period than following treatment with adult marrow of the same genetic combinations. Similarly, good survival was observed in strain combinations of the H-2^{k} group following protection with fetal tissue. In combinations in which there was a gene difference at the H-2 locus, the long-term survival was usually better following treatment with fetal tissue than with adult marrow but never reached as high as 50 percent. Since this mortality represents a major obstacle in the use of fetal hematopoietic tissue as a therapeutic agent, a study is underway, in an attempt to determine whether this mortality results from an immune response and its relation, if any, to secondary disease.

2. In certain strain combinations an anemia was observed following initial recovery from lethal radiation. Unlike the transient anemia mentioned above, this anemic condition is quite lethal. In collaboration with Commr. William McFarland, National Naval Medical Center, an attempt was made to determine the cause of this anemia. In testing animals of different genetic combinations as they became available it became apparent that this was not a simple problem. The types of anemia detected were hypochromic anemia, hemorrhagic anemia resulting from slow blood leakage into the small intestines and kidneys, and a possible hemolytic anemia; this latter diagnosis has not yet been confirmed. The possibility exists that this late type of anemia may result from an immune response

Part A.

initiated by a red cell antigen not governed by the H-2 locus which has been described for some inbred strains of mice. The genetic tests have not yet been undertaken to check this hypothesis. The complexity of this problem of anemia and the lack of technical assistance made necessary an indefinite postponement of any further hematological determination. The genetic studies will continue in an attempt to have available more definite information of a genetic nature at such time as this study may be resumed.

C. Preclusion of the homologous (secondary) reaction

1. The preclusion of the reac tion following treatment with Amethopterin gave hope that this might be a genetically non-specific method which might have practical clinical application. Other drugs tested were found to be too toxic in the irradiated and protected mice to be able to determine their efficiency in blocking the immune response under the experimental conditions used. Among the drugs tested were thioguanine, 5-fluorouracil and 6-azauracil. Cortisone and aureomycin were found to be ineffective in a limited number of trials. Since time-dose relationships have been shown to be very important, the negative results thus far obtained from drugs other than Amethopterin should not be considered all inclusive since very minor alterations in the treatment schedule have proved to be the difference between success and failure of the treatment. Further work is planned.

2. In collaboration with Dr. Raymond Popp, Biology Division, Oak Ridge National Laboratory, tests were made on irradiated animals treated with homologous marrow and one of the various drugs listed above to determine by their electrophoretic hemoglobin pattern if the graft was destroyed as a result of the drug treatment. In a sample of twelve mice ranging

Part A.

in age from three to twelve months post irradiation all hemoglobins were of donor-strain origin. This would indicate that the drug action is not one of slow destruction of the graft and recovery of the hosts hematopoietic system but probably one of a direct effect upon the immune response of the graft against the host.

3. Since the irradiated and protected mouse tolerated the drug treatment less readily than the non-irradiated animal and these treated animals were demonstrated by their hemoglobin patterns and tumor transplantation tests to be chimeras a somewhat different approach at drug treatment was attempted. It appeared that Amethopterin in the presence of an antigen permanently blocked the immune response against this specific antigen. An attempt was made to produce tolerant marrow donors by means of treated and non-irradiated mice with Amethopterin and injections of foreign spleen cells in various combinations. When these pretreated animals are used as marrow donors in the same strain as that of the spleen cells used in the pretreatment, the results were not easily explained. Under some pretreatment schedules, the marrow donors appear to have been sensitized against antigens of the host strain causing a more severe reaction than was expected from that particular combination although the early deaths described by others in presensitization experiments did not occur. Under other conditions there appeared to be prolonged survival although as yet an actual preclusion of the reaction has not been observed. In lieu of more promising methods of altering the homograft reaction, further attempts will be made using a slightly altered treatment schedule.

1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

D. Chimeras

1. The previously reported study of the histocompatibility patterns of the malignant lymphomas arising in the irradiation induced chimeras was continued. The more recent transplantation tests involved the high leukemic AKR strain as either marrow donor or irradiated host to the exclusion of those of other strain combinations. The experiments involving the AKR strain will be discussed elsewhere in this report; only the transplant data will be given here. Of the positive transplantation tests for homologous marrow chimeras, the neoplasms proved to be of donor-tissue origin in 49 cases and of host-tissue origin in 11 cases. In the chimeras produced by homologous fetal tissue inoculation, 15 neoplasms were of donor tissue origin and 2 of host tissue origin. A smaller sample of tests were conducted on animals receiving drug treatment (C 1). Six of these neoplasms were of donor tissue origin and one of host tissue origin, thus corroborating the finding of Dr Popp (C 2), that these mice are true chimeras in a second strain combination. The neoplasms involved in these tests were primarily lymphocytic and frequently involved the thymus. In approximately one-fourth of the tests the transplanted tissue failed to grow. These negative tests included several plasma-cell neoplasms and four adenocarcinomas of the small intestine which were transplanted as possible plasma-cell neoplasms. Adenocarcinomas of the intestines occur in untreated mice but are rare. The number found in this sample, which may be a very small percentage of those actually occurring in these treated animals, represents an increased incidence of this neoplasm. Several other neoplasms of unusual types were observed

Part A.

in this small sample of treated animals. Diagnosis has not been completed, however; they are considered by Dr. Thelma Dunn to be similar to those observed to occur in mice treated at birth with the Polyoma virus. No routine attempt has been made to demonstrate the presence or absence of a viral etiology for these neoplasms.

2. Reversal of the functioning donor type hematopoietic system to that of the host type is known to occur with uniform regularity in animals protected with heterologous marrow. There has been little evidence that 'reversal' occurred in animals receiving homologous marrow therapy. The occurrence of a single case of 'reversal' of homologous chimera led to the postulation that the greater the genetic difference between the host and donor the greater the probability of 'reversal' occurring. Of the 14 host type tumors observed in these transplantation experiments all represented marrow donors and irradiated recipients of the same H-2 phenotype, i.e., either both $H\text{-}2^d$, or $H\text{-}2^k$. These data would indicate that the prerequisite for 'reversal' need not be a 'great' genetic difference between host and donor.

B. Transfer of immunity by marrow graft

In collaboration with Dr. Victor Haas of NIAI experiments are in progress attempting to transfer immunity to the LCM virus from one mouse to another by means of lethal total body X-irradiation and isologous marrow inoculation. Although the experiments are still incomplete, it would appear that under these experimental conditions it is not possible to transfer immunity to the living antigen.

Serial No. NCI 427 (C)
1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

F. Multiple polyposis of the colon, a genetic trait

The development of a line of C57BL/10 H-2^d carrying the genetic trait for multiple polyposis of the colon was continued. Attempts at producing a malignancy from this benign lesion by irradiation were not particularly successful due to the high incidence of malignant lymphomas induced in this strain by the irradiation.

Serial No. NCI 42T (c)
1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part B.

Honors, Awards and Publications

Uphoff, D. E. Alteration of homograph reaction by A-methopterin in lethally irradiated mice treated with homologous marrow. Proc. Soc. Exp. Biol. & Med., 99; 651-653, 1958.

Uphoff, D. E., Law, L. W. An evaluation of some genetic factors influencing irradiation protection by bone marrow. J. Nat. Cancer Inst. 22: 229-241, 1959.

Fellow of the American Association for the Advancement of Science.

Serial No. NCI 427 (d)
1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

A study of the etiology and chemotherapy of experimental lymphomas

Delta E. Uphoff and Lloyd W. Law

Man years (calendar year 1959):
Total: 1 1/3
Professional: Uphoff, 1/2: Law, 1/3
Other: 1/2

A. Etiology and pathogenesis of experimental lymphomas

1. Attempts at altering the incidence of leukemia in the high leukemic AKR strain were extended to include the use of marrow of the four available H-2[k] strains and treatment at ages ranging from three to eight months. Although secondary disease occurs between 30 and 90 days following lethal irradiation and homologous marrow inoculation, an appreciable number of animals survive making possible a study of the incidence of leukemia in these chimeras, as well as the effect of varying degrees of reaction upon this incidence. The latter may prove to be an important factor since one of the results of a severe reaction is the depletion of the lymphoid elements. Although this study is still incomplete and no general statements may be made on the over-all incidence of leukemia in these animals, at this time some interesting observations can be made. Animals which are three to five months of age at the time of treatment may die as a result of the treatment e.g. from secondary disease , or may recover and eventually die of leukemia or from some other cause.

Part A.

Of those developing leukemia from transplantation tests the indications are that these neoplasms are primarily of donor tissue origin even though the donor marrow may be of a low leukemic strain e.g. C3Hf. A few neoplasms proved to be of host tissue origin and are considered "reversals". These are discussed elsewhere in this report. By six months of age some untreated AKR mice, particularly the females, may be expected to succumb to leukemia. Mice six to eight months of age with advanced lymphocytic leukemia of thymic origin at the time of irradiation may die shortly after irradiation apparently as a result of the toxic effects of the destruction of large masses of tumor tissue. A percentage of the animals which survive the acute irradiation damage will die of a "typical secondary disease". Other animals which succumb exhibit an unusual type of liver damage, the cause of which has not been determined. During the second and third months post-irradiation a few mice die of leukemia. Transplantation tests show these to be of host tissue origin. Unlike those previously mentioned, these are considered to be animals which were preleukemic or in the early stages of the development of the disease at the time of irradiation. The malignant cells were not completely destroyed by the irradiation and went on to develop a neoplasm which eventually killed the animal. This might be expected since a dose of 700r has not been demonstrated to be sufficient to destroy most neoplasms. Some mice which survive these episodes may live long enough to develop leukemia with a transplantation pattern similar to that described for the animals which are younger at the time of irradiation. Of these mice

Part A.

that develop leukemia, the age at death appears to be the same regardless of the age at treatment i.e., the older the animal before treatment, the shorter the latent period. In the reciprocal transplant i.e., AKR marrow in one of the four irradiated hosts, the incidence of leukemia is relatively low. A few of these neoplasms have been transplanted and were shown to be of donor tissue origin as might be expected. The high incidence of leukemia of donor tissue origin in the irradiated AKR mice and the "low" incidence of leukemia in the reciprocal combinations might indicate that rather than an indirect inductive effect of the irradiation as a causative agent, there may be fundamental differences between the AKR strain and the other four strains of the $H\text{-}2^k$ group. To test the role of the thymus in the induction of these neoplasms, thymectomized AKR mice will be treated with marrow of the Street strain in a manner similar to that of the intact mice.

2. The study of the effect on the incidence of lymphomas of exposure to 400 r, 100 r, and 25 r at birth with and without bone marrow inoculation at the 400 r level and 400 r at 30 days of age was completed. Intraperitoneal inoculation of C57BL marrow following 400 r at birth appeared to have little effect on the incidence of lymphomas, while an intravenous inoculation appeared to decrease the early neoplasms. Pathological analysis has not been completed making further generalizations impossible at this time.

3. The study of the effect of bone marrow and fetal hematopoietic tissue on the incidence of malignant lymphomas in intact and thymectomized C57BL and (C57BL×A)F_1 hybrid mice receiving 4 exposures of 168 r at 7 day intervals and treated with C57BL

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1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

marrow or fetal hematopoietic tissue was continued. In the F_1 hybrids at 13 months of age, the incidence of reticular neoplasms in the controls was 64%; in the fetal tissue inoculated group 34%; and following marrow inoculation, 22%. Similar results were observed in the C57BL strain (85%, 40% and 30%, respectively). Transplantation tests of the neoplasms arising in the hybrid mice indicated that the majority of the neoplasms were of host tissue origin. These results might indicate a lack of protection by the inoculated tissue although the occurrence of an occasional neoplasm of donor tissue origin and the time of onset would indicate the role of other factors. The differences in response of the X-rayed mice receiving marrow and fetal tissue may result from differences in the population of cells introduced. The earlier appearance and higher incidence of neoplasms in the mice receiving fetal tissue may reflect a potential inductive capacity of the embryonic hematopoietic tissue.

4. The previously proposed project to study the influence of complete and incomplete matching of the histocompatibility phenotypes on the prevention of X-irradiation-induced lymphoma has not yet been attempted because of a lack of animal space and technical assistance. A somewhat less extensive project was temporarily substituted for the proposed study in an attempt to determine the influence of the H-2 locus on the prevention of the induction of lymphomas in C57BL/Ka mice.

A single gene difference at the H-2 locus ($H\text{-}2^b$ versus $H\text{-}2^d$) does not prevent lymphoma induction in thymectomized C57BL/Ka mice, whereas $H\text{-}2^b$ bone marrow from C57BL/10 (differing at loci other than H-2) is effective in preventing the induction.

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1. (Laboratory of Biol[illegible])
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

Embryonic hematopoietic tissue (C57BL/Ka), though strikingly effective in producing immediate thymic regeneration, is not (unlike adult bone marrow) very effective in reversing the carcinogenic process. These studies are continuing in the following aspects:

a) Study of the dose-response effects of embryonic hematopoietic tissue

b) Influence of other H-2 allels such as other H-2^b, H-2^d, H-2^k, H-2^a, using both embryonic and adult hematopoietic tissues.

B. Chemotherapy of transplantable leukemias

Attempts at treating drug toxicity in tumor bearing as well as normal mice by bone marrow inoculation was continued. A series of AKR mice were treated with therapeutic dose level of thioguanine and an intravenous inoculation of C3Hf bone marrow. The leukemias arising in these treated mice were tested by transplantation and were found to be of AKR tissue origin. This would indicate that transplantation of marrow under these conditions was either not successful or that 'reversal' had occurred in virtually 100% of the animals treated. With the possibility existing that in these animals receiving antimetabolites and homologous marrow, a state of immunological non-reactivity (or 'tolerance') toward the strain of the marrow donor may exist, a second group of mice will be treated and tested by skin grafting. This is an important adjunct to the attempts at precluding the graft-host reaction in lethally irradiated mice treated with homologous marrow by the use of antimetabolites.

Serial No. NCI 427 (d)

1. (Laboratory of Biology)
2. (Leukemia-Studies Section)
3. (Bethesda 14, Md.)

Part A.

C. Radiation therapy of transplantable neoplasms of reticular cells

Following the report of Loutit and colleagues at Harwell of sensitivity of one malignant lymphoma to high level X-radiation, protracted over 24 hours, we undertook a study of the response of all available morphologic forms of reticular neoplasms (a total of 10). Striking responses were obtained with a type B neoplasm (P195) and with a type A neoplasm (L11371). Work is continuing with the latter, a chronic neoplasm in C57L mice to study the influence of dose rate and protraction of irradiation, of the effect of isologous versus homologous bone marrow following irradiation and the permanence of response in later transfer generations.

Part B.

Not included.

1. [illegible]
2. Viroma Cultures Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Microcinematographic studies of tissue cells in vitro.

Principal Investigators: W.T. McQuilkin, W.R. Earle.

Other Investigator: V. J. Evans.

Cooperating Units: None.

Man Years (calendar year 1959):
Total:
Professional:
Other:

Patient Days (calendar year 1959):

Project Description:
1. A study of the alteration of morphologic and behavioral characteristics of strain NCTC-929 (strain L) cells resulting from growth in chemically defined, protein-free medium, NCTC-109.

Objective: To determine quantitative and qualitative differences between the morphologic and behavioral characteristics of cells of this well known strain when it is maintained in its accustomed protein medium and after it has been adapted to the nutritional environment of the chemically defined protein-free medium NCTC-109.

Methods Employed: Detailed study and analysis of many time-lapse motion pictures of both the parent and the adapted cell strains. Quantitative time data is obtained by correlating the frame frequency with the observed occurrence of events in the film.

Part B included Yes x No

Major findings: Some quite definite alterations have occurred in the cell strain as a result of adaptation to and establishment in the chemically defined protein-free medium. Quantitatively, the average generation time is about 1/3 longer; the rate of migration of cells on the glass substrate is reduced by at least 90 percent, and the duration of the cytokinetic period has been estimated tentatively to be about 1/5 as long. Qualitatively, the cells show a less compact, less refractile and less granular morphology; cyclosis is altered to a more peristaltic type motion; the cells maintain a more independent existence with a decreased affinity for each other and consequently appear to have less mutual influence; cells do not intermittently round up and spread out as formerly, but almost exclusively round up only for the prophase stage of mitosis; cytokinesis is not only of shorter duration, but occurs in a much less violent manner. No statistically significant findings of cell death have been observed in either cell-medium system. However, a few spectacular cell deaths by complete and sudden disintegration have been observed in films of one subline of the derived cell strain.

2. Analysis of film of a strain of monkey kidney cells maintained on chemically defined, protein-free medium.

Objective: To determine the proliferation rate in this cell-medium system.

Major findings: The average generation time of this epithelial cell strain is approximately 37 hours, which is comparable to the previously determined time of fibroblast cells in this same medium. This rate of proliferation indicates a population increase of approximately 24-fold in a 7 day period.

Significance: These studies indicate the methods that are being employed and the efficacy of the cinematographic technique as an instrument of research in population dynamics of living cell cultures in vitro.

The first study, above, characterizes some of the factors of cell morphology and cell behavior of an established in vitro cell strain of mouse fibroblasts that cannot be determined in any other way. It further elucidates some alterations that have occurred in this cell strain as a result of a radical change in the nutritional environment.

The second study, above, confirms the rapid proliferation rate potential of the important monkey kidney strain when grown in vitro in chemically defined, protein-free medium NCTC 109. It also demonstrated that successive generations of particular individual cells can be studied cinemicrographically (in this case up to six generations).

Proposed course of project: To continue the present line of investigation of the alteration in proliferation rate, morphology and cellular behavior in cell strains, as correlated with oncological transformations, viral activity, and nutritional changes. It is proposed that refinements and extensions of observations will be made by the addition of the techniques of higher magnifications, phase microscopy, and perfusion.

Part B: Honors, Awards, and Publications.

Films have been provided, by request, to the British Broadcasting Co., for use in a forthcoming science series TV broadcast entitled "The Last Scourge".

Data from 2, above reported in article "Growth in vitro of a long term strain of monkey kidney cells in medium NCTC 109 free of any added protein; " published in American Journal of Hygiene, Nov. 1959, (see section 428-C).

NCI
Serial No. 428(b) - Project 1
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part A

1.
Project Title: Rapidly agitated fluid suspension cultures for the growth of cells of various established cell strains.

Principal Investigators: J.C. Bryant, E. L. Schilling, W.R.Earle.

Other Investigators: None.

Cooperating Units: None

Man Years (calendar year 1959):
Total:
Professional:
Other:

Patient Days (calendar Year 1959):

Project Description:
Objectives: To continue attempts, described in last year's report, to define and control more closely the factors influencing growth of mammalian cells in rapidly agitated fluid suspension cultures. Emphasis this year has been placed on 1) adaptation of new cell strains to this type of culture, and 2) developing and testing of a new gas-metering device for controlling the flow of gas through the cultures above the fluid.

Methods Employed: These have been described in earlier reports: special 1½ liter shaker flasks containing a maximum of 400-450 ml. fluid, continuously aerated through the gas phase, and agitated on a shaker platform at 8640 revolutions per hour (rph). Nuclei counts and reducing sugar analyses were made regularly on small aliquot samples.

Part B included Yes x No

The old gas-metering device (Earle, et al., Annals N.Y. Acad. Sci. 63(5): 666, 1956) was replaced early during the year by a similar and more accurate electronically controlled gas metering system. (See 428(f). The gas phase of each agitated culture receives one short burst of gas during each 15-second interval. This can be varied at will. Three different gas mixtures have been used: a) 5 percent CO_2, 25 percent O_2, and 70 percent N_2; b) 5 percent CO_2 in air; c) 10 percent CO_2 in air.

Major Findings: The kind of gas mixture used, of those listed above, had no effect on the proliferation rate of the cell strains studied, either a monkey kidney cell strain NCTC 3196 (LLCMK2) or a mouse fibroblast strain 2071. The medium was protein-free chemically defined medium NCTC 109 supplemented with Methocel.

The successful demonstration during the two previous years that established cell strains such as NCTC clone 929 from strain L and NCTC liver clone 1469 could be repeatedly carried through short harvesting-subculturing cycles for high net yields at high population levels made it advisable this year to extend the procedures to other cell strains, such as strain NCTC 2071 from clone 929 mouse fibroblasts, a monkey kidney cell strain, and a human skin strain. The elimination of serum and the substitution of methylcellulose as a supplement to chemically defined medium NCTC 109 has been given major emphasis. Project 2 of Serial No. 428(b) reports the studies of the factors involved.

Significance: Use of these methods for cultivating mammalian cells in agitated fluid suspension cultures makes possible the production in vitro of large amounts of cells of various strains, both normal and malignant, under much more accurately controlled experimental conditions. Consistent availability of large quantities of cells of a given strain at frequent intervals and under rigidly controlled experimental conditions for long term biochemical studies is now of major importance in many lines of research.

Proposed Course of Project: The procedure may be extended to other selected strains of cells which are important for cancer research. A major long-term objective is to determine the conditions necessary for maintaining a rapid aerobic harvesting-subculturing cycle for various cell strains. Improvement of the procedures is a continuing objective. The procedure will be extended to incorporate a continuous flow apparatus for continuous growth of the cells under steady state conditions.

Part B. Honors, Awards, and Publications:

(Publications other than abstracts from this project:)
(Honors and awards:)

A paper presented April, 1958 by invitation at the "Symposium on Cultivation and Use of Animal and Plant Cells", an Interscciety Symposium of the Federation of American Societies for Experimental Biology, Philadelphia, Pa., was based in large part on this project and presented current results from this project. The paper was published in amplified form as follows: Earle, W.R. : Long-term cultivation of animal tissue cells in large cultures. Fedn. Proc. 17(4): 967-974, 1958. Publication was too late to be included in our 1958 annual report. See Earle project 428(f) for listing.

NCI
Serial No. 428 (b) - Project 2
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project 2, Title: The growth-potentiating effect on chemically defined medium NCTC 109 of Methocel with certain cell strains in a) rapidly agitated fluid suspension cultures and b) small static cultures.

Principal Investigators: J. C. Bryant, V.J. Evans, E. L. Schilling, and W. R. Earle.

Other Investigators: B. B. Westfall, E.V. Peppers.

Cooperating Units: (Project 2b) Navy Tissue Bank, Naval Medical Center, (P.C. Bakken).

Project description:
Objectives: To grow mammalian cells in agitated fluid suspension cultures in chemically defined medium NCTC 109 without serum supplement by substituting a non-protein chemically defined Methocel. Emphasis is placed on a monkey kidney cell strain.

Methods Employed: The type of culture and the basic procedures are considered in Project 1, Serial 428 (b).

Major Findings: Exploratory investigations reported last year, mouse fibroblasts of strain NCTC 2071 in different types of culture flasks, various grades and concentrations of Methocel, and with a silicone coating on the walls of the flasks, were confirmed this year. As an example, in two cultures of one experiment, strain 2071 cells proliferated for 17 days at a moderate logarithmic rate expressed as 4.86 days and 4.68 days respectively average generation times. Population increased from 81 and 63 million cells in 100 ml. to 911 and 785 million cells in 400 ml. Repeated harvesting cycles, however, have not yet been attained for this strain or for any other cell strain

Part B included Yes x No

in this medium in agitated cultures.

Many experiments have been carried out in agitated fluid suspension cultures with the monkey kidney cell strain NCTC 3196 ((LLCMK2 from Lilly Research Laboratories). This strain has been proliferating rapidly in static cultures in protein-free chemically defined medium NCTC 109 for 14 months (See Evans project 428(c). Generally the agitated fluid suspension cultures of these cells have presented an anomaly: the cells remain healthy in appearance as either single cells or small irregular loosely packed clumps of cells, but show little if any increase in numbers over periods of time up to two months.

In a few cases these monkey kidney cell cultures have shown slow proliferation. For example, one culture on NCTC 109 medium supplemented with a low viscosity Methocel (25 cps) at 0.12 percent gave a four-fold increase in 21 days. In another experiment where Methocel supplement was at much higher concentrations (25 cps at 1.65 percent and 4000 cps at 0.45 percent) two cultures increased in population from about 28.5 million to 127 million, in 75 to 80 ml. fluid, a 4.5-fold increase in 34 days. This similarity in proliferation rates between these two experiments occurred despite a wide difference in actual viscosities of the complete medium; the relative viscosities were 1.3 in the first case and about 10.0 in the second experiment. This appears to eliminate viscosity of the medium as a significant factor.

Other factors studied in many experiments with monkey kidney cells in agitated fluid suspension cultures include the following:
1) Doubling the concentration of 13 amino acids of NCTC 109, arg, cystine, glu-NH_2, hist, iso, leu, lys, meth, phe, thr, tryp, tyr, and val, had no discernable effect.
2) Methocel viscosity grades tested, 10, 15, 25, and 4000 cps, showed no consistent discernable effect. The few cases where slow proliferation did occur were with 25 cps and 4000 cps Methocel.
3) The concentrations of Methocel tested were principally 0.06 and 0.12 percent of the low viscosity grades, but included in some experiments higher concentrations up to 1.6 percent of 10 cps. Concentration of Methocel had no apparent correlation with cell population.
4) Cell concentrations in the inoculum and in fresh fluid at each fluid change were tested at 500,000/ml, 1 million/ml, and 2 million/ml. These was no discernable effect.
5) The effect of two different glucose concentrations in the fresh medium, 100 milligram percent (normal for Earle's saline and for medium NCTC 109) and 26 mg. pct., was examined. The higher concentration of glucose in the fresh medium was accompanied by an increased rate of glucose utilization but not by any increase in cell proliferation rate.
6) Horse serum as supplement (20 percent) was less effective than were Methocel supplements: the cultures disintegrated within a short interval of time, whether in siliconed-coated flasks or in untreated flasks.

7) Determination just before each fluid change of total protein and total nucleic acids on aliquots samples of cell suspension by Dr. Westfall (project 428 (d)) showed a fairly close correlation between the values obtained and the nuclei counts. This correlation might be expected from the static non-proliferative nature of the cultures.

In addition, an exploratory experiment with monkey kidney cells in replicate roller tube flasks was carried out to estimate the effect of certain other variables. A 3-4 day fluid change interval gave much higher proliferation rates than did the 7-day interval. Methocel of 50 cps viscosity grade was slightly more effective than was 25 cps Methocel. Methocel at 0.12 percent concentration was slightly more effective than it was at 0.06 percent concentration. The highest drum speed, 2000 rph, was more effective than were either 1000 rph or 500 rph drum speeds. The indication that Methocel of higher viscosity grade and at higher concentrations was more effective was considered to merit further study.

A consequent exploratory experiment with static T-15 replicate culture flasks involved the use of six different viscosity grades of Methocel at several different relatively high concentrations for another strain of cells, human epithelial strain NCTC 2544 from clone NCTC 2414. These cells had previously been grown in chemically defined medium NCTC 109 in static flasks for several months only with considerable difficulty, and have recently been found to proliferate at a moderate rate in the same medium supplemented with Methocel in agitated fluid suspension cultures (Evans project 428 (c)). While the cells proliferated only slowly, all treatments did show some increase in cell numbers over the inoculum population. Methocel concentrations had no effect on proliferation. Of the six viscosity grades of Methocel tested, 10, 50, 100, 400, 1500 and 4000 cps, the 100 cps grade gave the highest proliferation rates, by a significant margin.

Significance: Since some strains of mammalian cells can now be either grown or at least maintained in massive agitated fluid suspension cultures, cell nutrition studies in the Tissue Culture Section (Evans project 428 (c), Westfall 428 (d)) with chemically defined media are now being extended from static cultures containing 3 to 15 ml. fluid each to agitated fluid suspension cultures containing 100 ml. to 400 ml. fluid each. Comparative studies of normal and malignant cells can be accelerated as it becomes possible to grow more cell strains in chemically defined media in agitated cultures.

Proposed Course of Project: The use of methylcellulose as a protective agent is being studied more intensively with the selected cell strains used so far, in order to attain rapid proliferation and frequent harvesting-subculturing cycles; this study will also be extended to other selected important strains of mammalian cells. Similarly potentially protective chemically defined non-protein agents such as dextrans and PVP (polyvinl-pyrrolidone) may be investigated for their effect on the growth of cells in chemically defined media in agitated cultures.

The mechanism of the protective effect of Methocel supplement under these conditions is being explored. The extent to which Methocel has a nutritive effect, if any, in addition to or complementary with its protective effect, needs elucidation.

Further planning for future extensions and applications of this study is based on the following facts. 1) Present deep-freezing methods for preserving cells include the use of glycerol as a protective agent; glycerol, while it is a known chemical entity, affords only limited protection and is at best inadequate; also, serum is present in culture fluid. To our knowledge, no long-term tissue cultures grown on chemically defined media have been subjected to freeze-drying studies. 2) Preservation of cells by freeze-drying will certainly be used to a greater degree in the near future because it may reduce the possibility of cell change during extended intervals of time, and because it may save time and labor. 3) Methocel has been shown in our laboratory to protect cells of certain strains in agitated and in static cultures, in the absence of serum, allowing the use of protein-free, chemically defined media. Therefore it is recommended that the use of Methocel instead of glycerol as a protective agent during and after freeze-drying, for cultures of cells which have been grown in protein-free chemically defined media, should be studied by authorized investigators.

Part B Honors, Awards, and Publications:

Publications other than abstracts from this project:

1) Bryant, J.C., Schilling, E.L., Earle, W.R., and Evans, V.J.: Massive fluid suspension cultures of certain tissue cells. IXT. Exploratory supplementation experiments with chemically defined medium NCTC 109. J. Nat. Cancer Inst. 23: 000-000, 1959. (In Press).

Paper IV of the series "The growth-potentiating effect of chemically defined medium NCTC 109 with Methocel on strain 2071 cells cultures in silicone-coated flasks" is in preparation.

Honors and awards relating to this project: None.

NCI
Serial No. 428 (c)- Project 1
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part A

Project 1, Title: Studies of Cell Nutrition in vitro.

a. Cultivation of Monkey Kidney Cells in protein-free medium NCTC 109 and their use in virus research.

b. Cultivation of fresh C3H mouse fibroblasts in chemically defined medium.

c. Cultivation of a long term strain of human skin epithelial cells in medium NCTC 109, a chemically defined medium free of added protein.

d. Studies on simplification of chemically defined protein-free medium NCTC 109.

Principal Investigator: V.J. Evans.

Other Investigators: J.C. Bryant, W.R.Earle, W.T.McQuilkin, H.A. Kerr, G.A.Parker.

Cooperating Units: Lilly Research Laboratories; Tissue Bank, Naval Medical School; Dr. E. Shelton, Cellular Biology Section.

Man Years:(calendar year 1959)
Total:
Professional:
Other:

Patient Days (calendar year 1959)

Project description: a) Cultivation of monkey kidney II in medium NCTC 109 a protein-free chemically defined medium.

Part B included Yes x No

Objective: To determine if it is possible to grow monkey kidney cells in long term cultivation in protein-free chemically defined medium, so that such strains of cells can be used as laboratory tools and model cell types for virus studies and metabolism studies.

Method employed: Strain MK2 - Lilly Research Laboratory - was immediately transferred (after receiving them in this laboratory) from a medium containing Medium (Morgan) 199 and 1% serum to Medium NCTC 109. Fluid change schedules were varied to obtain desired populations and culture behavior.

Major findings: The cells have ultimately been satisfactorily cultured in all sizes and types of culture flasks used in the laboratory. Fluid removal schedules were worked out for optimum use of cultures for virus assay studies particularly for polio virus. Rates of growth were determined on the microcinematograph. A report in November issue of American Journal of Hygiene gives details of titer of polio virus grown in the cells as well as details of cultivation and behavior. A clone strain on this medium has also been obtained and is well established in T-60 cultures.

Significance: This is the first long-term strain of monkey cells carried in vitro on a completely chemically defined medium for over a year and still under continuous satisfactory cultivation in the medium. Already it has value in virus studies since polio virus Type III grows in it at as least as high titer as this virus will grow in fresh monkey cells. It is expected, as the information becomes more general by way of the published papers the strain will assume great significance for those investigators desiring a strain of cells free of "wild" viruses. The fact that serum is not required may make it additionally valuable for studies where serum presence may interfere in any way with virus cultivation as it well might in measles and mumps virus cultivation for vaccines.

Proposed course: Continuous maintenance of strain is proposed with the expectation of making it available to other laboratories for virus studies. It is in use for studies in the methodology of both cultivation of large agitated fluid suspension cultures and(in collaborative studies) in possible cultivation of viruses from human tumors.

Project description: b) Cultivation of fresh C3H mouse fibroblasts in medium NCTC 109, a protein-free chemically defined medium.

Objective: 1) To establish new strains of C3H mouse fibroblasts directly in chemically defined medium so that an attempt can be made to determine whether or not cell transformations to tumor-producing cells in vivo are influenced by the composition of the medium. 2) To induce transformations in vitro in such cells at the appropriate time.

Methods employed: Several series C3H mouse fibroblasts have been planted in vitro by one or the other of two methods. 1) Pooled minced embryonic mouse cells of varying types have been used. 2) Explants of small pieces of tissue from the epigastric fat pad have been retrieved from double millipore filter chambers and planted in vitro under finely perforated cellophane in NCTC 109 medium free of serum. Control cultures have been established in medium NCTC 109 with the addition of 10% horse serum by volume.

Results: One "strain" of cells from the pooled embryos has been in vitro 9 months in the completely defined medium. The cell type appears to be predominately fibroblasts. Growth is extremely slow compared with the control strain with the serum. To date no daughter generations can be established from it. Nevertheless, it is alive and growing at least in amounts satisfactory for exploratory micro injection work. Each new series has been maintained longer than its predecessor, indicating definite progress toward the desired objective. The exploratory studies with tissue adapted in vivo in the double millipore filters prior to retrieval and implantation in vitro in the chemically defined medium may have more promise of continuous rapid cultivation than that from pooled embryonic tissue. These cultures morphologically appear like all fibroblasts grown in vitro, and seem, at least after a short period, to be "holding their own" in the chemically defined medium.

Significance: Continued success appears to be far more likely now in cultivation of fresh tissue in chemically defined medium; attainment of the ultimate objective for the cultivation is definitely hopeful.

Proposed Course of Project: 1) To continue with the strain available; 2) To develop others for continuation of and to improve medium and methodology of cultivation; to pursue as rapidly as possible with them the obvious objectives of the cultivation of fresh tissue in chemically defined medium. (See section 428 (c), project 2) for explanatory and preliminary results on the general objective.)

Project description: c) Cultivation of a long term strain of human skin epithelial cells in medium NCTC 109.

Objective: To determine if it is possible to grow human skin epithelial cells in the protein-free chemically defined medium NCTC 109 in static cultures.

Method employed: Cells of the clone strain NCTC 2544 of human skin carried in NCTC 109 with 10% human serum were gradually depleted of serum. The first reduction was to 5% serum and then to 0.125%. Partial fluid renewals were also used initially in this adaptation to retain population. Since the cells became free floating on this regime centrifugation prior to fluid renewal was required. Periodic return to the small volume of serum was alternated with cultivation on the complete NCTC 109 with no serum until finally cultivation on

serum free medium with no periodic returns to serum containing medium was possible.

Results: A long-term strain of human skin epithelial cells has been adapted to routine cultivation in a chemically defined medium free of added protein. The growth rate is relatively good.

Significance: This is the first long-term strain of human cells ever to be grown routinely and continuously in a protein-free chemically defined medium. The potential of these cells for many aspects of virological and cancer research, is considerable.

Successful epithelial cell transplantation for either a temporary dressing, or a longer term one, or as a stimulant to the recipient's own cells has a clinical bearing on reparative surgery in general and on burn problems in particular in military medicine.

Future Course: To pursue at a minor exploratory level the potential growth of these skin cells in mass amounts in fluid suspension cultures in chemically defined medium; (See Series 428(c),project III, Chemically defined medium) to make the breadth of our knowledge on the cultivation of this strain of cells available to those who might propose to use the cells as an integument or integument substitute and also to the virologists for their use particularly with respect to possible cultivation of possible human tumor viruses.

Project description: d) Studies on simplification of medium NCTC 109.(See Dr. Sanford's report also.)

Objective: To simplify composition of medium to the maximum without sacrifice of quality or quantity of cells.

Methods employed: Replicate cultures of strain NCTC 2071 (strain L) mouse fibroblasts followed by enumeration of nuclei of each culture in each set to determine the effect of proliferation of individual or combinations of components. Long-term cultivation of cells on media that have been designed as results of findings from exploratory studies by replicate culture procedures.

Results: Medium NCTC 117 has been formulated and put to prolonged test. This medium NCTC 117 has been derived from medium 109 by omitting coenzymes, sodium glucuronate, deoxyguanosine, deoxyadenosine, and 5-methylcytosine, and "essential" unsaturated fatty acids.

Significance: Medium NCTC 117 is simpler and substantially cheaper to prepare than medium NCTC 109. Further it has been demonstrated that apparently for both excellent quantity and quality of cells the only nucleic acid derivatives required for strain L are thymidine and deoxycytidine. It has also been revealed that adenosine triphosphate is not required by these cells; this suggests that they must have some mechanism as yet undiscovered for producing it.

Future course of project: To complete exploratory studied on the necessity for the so-called combination of reducing substances, (glutathione, cysteine and ascorbic acid) in medium NCTC 109. To collaborate with Dr. Sanford (as the result of her findings on the vitamin studies) on redesigning a completely simplified chemically defined medium for strain 2071 (strain L) cells. To use findings on, strain 2071 mouse fibroblasts (as prototype cell) to evolve better media for fresh tissue and for cell strains so far not grown in chemically defined medium. To use simplified medium in the first intermediary metabolism studies done with cell strains grown in vitro in chemically defined medium free of the interference from and possible influence of added protein. Furthermore, for example, experiments are now being planned to study the possible adaptation to protein-free chemically defined medium NCTC 109 of certain cell strains which have been previously intractable to such medium. Such a cell strain is liver clone NCTC 1469. The supplements to be used in an attempt to effect adaptation include these groups: 1) Methocel and spermine, 2) pyruvate, oxalacetate and alpha-keto glutarate, and 3) serum as a control. Spermine has been found by Mager (Nature 103: 1827, 1959) and others to influence cell permeability of certain bacteria and might prove to influence similarly mammalian cells. Special nutritional needs of the intractable strains might possibly be met by the three key metabolic intermediates.

Part B Honors, Awards, and Publications.

Publications other than abstracts from this project:

1) Evans, V.J., Fioramonti, M.C., and Earle, W.R.: Studies of nutrient media for tissue cells. V. The effect of the coenzymes of medium NCTC 109 on the proliferation of NCTC strain 2071 and NCTC strain 2937. Am. J. Hyg., 70: 28-33, 1959.

2) Evans, V.J., Kerr, M.A., McQuilkin, W.T., Hull, R.A., and Earle, W.R.: Growth in vitro of a long-term strain of monkey kidney cells in medium NCTC 109 free of any added protein. Am. J. Hyg., 71: 1959.

Honors and awards relating to this project:

Continued as member of General and Cellular Biology Review Panel. (Evans)

Continued as Consultant to Navy Tissue Bank, U.S. Naval Medical School, National Naval Medical Center. (Evans)

By invitation presented paper at Atlantic City April, 1959 in the Tissue Culture Association Symposium on the Evolution of Cell Population. Title of paper: Large scale long term cultivation of cells in chemically defined medium. (Evans)

Presented paper by invitation before Committee on Cell Culture in a conference sponsored by the Institute of Laboratory Animal Resources and the National Research Council held at the National Academy of Sciences January, 1959. Subject of paper: Nutritional Standards for Cells in Tissue Culture. (Evans)

Participated by invitation in meeting at Wilson Hall, May 1959 on Characterization, Preservation and Supply of Tissue Culture Cell Lines, sponsored by Viruses and Cancer Study Section Panel Research Grants Branch, National Cancer Institute. (Evans)

NCI
Serial No. 428 (c) - Project 2
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project 2 - Title: Cell transformations in vitro.
a) Carcinogenesis potential of tissue culture media.

b) Studies on fresh C3H mouse tissue in chemically defined media both with and without the addition of some horse serum.

c) Studies on fresh C3H mouse tissue in chemically defined medium for carcinogenesis.

Principal Investigators: V. J. Evans and G.A. Parker.

Other Investigators: W. R. Earle, and Ann Palko.

Cooperating Units: None

Man years:
Total:
Professional:
Other:

Patient days (Calendar year 1959)

Project description: a) Carcinogenesis potential of tissue culture media.

Objectives: To investigate the possibility of tumor production from injection of 1) the standard chick embryo extract -horse serum medium. 2) Single members of this mixture, 3) Unfiltered chick embryo extract. 4) Chemically defined medium NCTC 109, 5) Used filtered supernatant fluid from mouse liver cells in vitro.

Part B included Yes No X

Methods employed: All the above media injected into several hundred newborn NIH Swiss, C3H x AKR, and C3H mice. Injected animals are being kept one year unless moribundity supervenes.

Results: A majority of animals have reached the terminal period of experimental plan. Gross examination reveals no clear cut indictment of any particular variable. Histological preparations have been returned on a large number of animals. Observations have been to date on the Swiss and hybrid strains. Frequent anomalies have been revealed in the lymphatic tissues, kidney, liver and salivary glands. Several cases of enlarged spleen and lymp nodes have been seen. Heavy infiltration of lymphocytic cells has occurred of salivary glands, kidney and liver . The findings so far have yet to be finally correlated to find out if any neoplastic change is associated with any specific type of fluid injections into any of the strains of animals.

Significance: It is hoped that the data obtained from injecting the 3 strains of animals with the aforementioned media and their components will supply base-line knowledge to the phenomenon of cell transformation in vitro and/ or tumorigenesis in long term tissue culture. Resolution of this problem will aid in answering the question of why cells transform after continuous cultivation in vitro. It will also make long term tissue culture strains of additional significance in industrial application as related to chemotherapeutic testing and other fields.

Proposed course of project: To continue this study as originally planned. Should it be ascertained that the media or any of their components contain agents that are capable of producing pathological and/ or neoplastic changes when injected into newborn animals, then a fertile area of future study is open for the identification and isolation of the agents and for further studies on the control and elimination of such changes.

Project description: b) Studies on fresh C3H mouse tissue cultured in chemically defined medium supplemented with horse serum.

Objective: To determine the time the transformations occur in freshly isolated embryonic C3H mouse cells when they are cultured in vitro on chemically defined medium supplemented with horse serum.

Method employed: Tissue from 18-19 day old C3H embryos was minced and cultured in medium NCTC 109 + 10% horse serum. Routine establishment of two series of cultures (3143, 3403) by the usual laboratory procedures was carried out.

Intraocular and intramuscular injections of the tissue were made according to the schedule shown in the table in the results section.

Results: The following table on page 6 gives the results to date on intraocular implants. The data contained therein should be regarded as exploratory evidence subject to revision by results from a third series, still to be carried out, to define to a sharper degree the exploratory data so far obtained in the first two exploratory series where perfecting methods and techniques and obtaining guidelines were of primary importance. In addition to the data in the table some simple observational data on the behavior of implanted tissue in the eye has been collected. At the moment, they have little critical significance but they may be expected to assume importance as subsequent confirming data are collected: Implants of tissue 37 and 51 days in vitro rounded up into small discrete masses and the rest of the eye showed little reaction to their presence. Tissue 73 and 93 days in vitro upon injection invaded the iris and subsequently was resorbed. Tissue 121 days in vitro when injected spread in the eye and affected the pupil. Tissue 147 days in vitro became highly vascular and protrusion of the cornea occurred; occasionally the cornea ruptured. Tissue 168 days in vitro upon injection produced tumors within 17 days; these were largely sarcomatous, i.e.,the cells in the tumors are elongate fusiform cells and ovoid with many mitoses and definite infiltration in the normal tissue occurs.

INTRAOCULAR IMPLANTS OF C3H CELLS IN VITRO

Days in vitro	SERIES II		SERIES II	
	Tumors	TC Growth Acceleration	Tumors	TC Growth Acceleration
37			⊕	
51			⊕	
73			⊕	
93				
103				**
107			⊕	
121			⊕	
135		**		
147			⊕	
148			⊕	
168			⊕	
176	⊕			
198			⊕	
199	⊕			
224	⊕			
262	⊕			

*This datum is based on judgement of when culture growth "appeared" accelerated.

Significance: These exploratory data show that cells cultured in vitro have the capability (by the injection procedure used) of producing tumors in the eye in series I within 5-6 months after explantation and series II within 4-5 months. In series I the transformation appears to have occurred within a 28 day interval and in series II within a 26 day period. This is remarkably close corroboration of the two and suggests that perhaps more extensive injections must be made on cultures from the 3rd to 6th month of their life in vitro in order to determine if the 28 and 26 day interval must be further narrowed. It would seem that this information is necessary in order to know the period of time during which investigations may be made to determine the bio-dynamics of the transforming phenomenon. These data suggest many leads. First it will be observed that accelerated growth of the cultures occurs prior to the time in the life of the culture when the cells will on injection produce intraocular tumors. At the moment there is no way of knowing absolutely whether this has causal significance or is merely parallel occurrence. If there is the concept that the transformation is associated with contamination by "a particulate agent" at a low titer then the interval of time of the cells in vitro might be regarded as the period required to raise this titer sufficiently to yield enough agent to produce the tumors. If the transformation is considered as resulting from having grown the cells under anaerobic conditions with concomitant alterations in cell metabolism then this period of growth in vitro is significant as the one in which such a transition has taken place. If it is possible that the capacity of the strain of cells to produce tumors on injection is related to a selection of cells in the population then consideration should be given to very early cloning studies and to confirmation of the data already obtained with the mixed populations. Furthermore now that the time of transformation is quite narrow, as revealed in these studies, some consideration might be given to the merit of a limited study of a few months on the dynamics of chromosome patterns in the cells, particularly if cloning studies are carried out and confirmation of mixed strain data is obtained on cloned strains.

Proposed course of study: To confirm results to date and pursue some hypotheses considered under significance.

Project description: c) Studies on fresh C3H mouse tissue in chemically defined medium for carcinogenesis.

Objectives: To investigate 1) Whether or not C3H mouse fibroblasts grown in chemically defined medium MCTC 109 with no blasts grown in chemically defined medium MCTC 109 with no added serum will produce tumors upon injection into the strain of origin and 2) if so after how long a cultivation time in vitro.

Methods employed: Tissue from 12-16 day old C3H embryos was cultured in medium NCTC 109 with no added serum. Intraocular injections were made of fresh cultured tissue and of tissue carried by the routine laboratory procedures for various periods of time.

Results: Several series of cultures have been attempted in the chemically defined medium. Each new one has been increasingly better growing than its predecessor. The current one has been in vitro over 9 months. Though hardly to be considered as a strain of cells it is possible that it will continue to grow and ultimately become one. Eye implants from cells of these several series have been made after the cells have been in vitro 26, 37, 51, 161 and 211 days. Everyone of these has resorbed. The rate of growth of the cells in all these series has been slow.

Significance: There is promise from the last series and hope for future series that a study (similar to that one described in part b), may be carried out with cells exposed only to nutrient having a completely chemically defined composition. If this is possible greater control can be obtained of culture conditions as they may effect transformation of cells in vitro. Such a study should reveal some information on the role of media in transformation.

Proposed course: Further eye implants from more series growing for a longer period of fresh tissue in chemically defined medium is planned. Establishment of growth rate microcinematographically. Following out some of the concepts advanced in part b of this project.

(3) - Project 3
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project 3, Title: Collaborations in Tissue Culture with other organizations.

a) Collaboration study on steroid enzyme activity of stabilized mouse, human liver and other cell strains in vitro.

b) Project description b- Collaborative studies on determination of relative antigenicity of long term strains of human skin cells and fresh skin cells.

c) Growth of human skin cells in chemically defined medium in rapidly agitated fluid suspension cultures.

d) Studies on the possible use of NCTC 109 as an intravenous feeding solution.

Principal Investigator: V. J. Evans.

Other Investigators: J. C. Bryant, E. L. Schilling, W.R. Earle.

Cooperating Units: a) G. Tomkins, NIAMD, Laboratories of Biochemistry and Metabolism.

b) M.A. Fink, Virus Oncology Section, NCI.

c) Navy Tissue Bank, Paul Bakken.

d) Dr. J. Wolstenholme, Veterans Hospital, Orange, N.J. and Navy Tissue Bank.

Part B included Yes No X

Man years (calendar year 1959)
Total:
Professional:
Other:

Patient Days (calendar year 1959)

Project description: a) Steroid enzyme activity of stabilized of mouse fibroblasts, mouse liver cells, human liver cells, human skin epithelium, monkey kidney cells.

Methods employed: Many but prime T-60 cultures of stabilized cell strains of mouse fibroblasts, mouse liver cells, human liver cells, human skin epithelium have been prepared by our methods of cultivating T-60 cultures for Dr. Tomkins for his analysis. (See his report for techniques used).

Results: The results are tabulated below. (See page 3)

IN STRAINS

Enzymes Reducing:	Mouse Liver 1469	Strain L 2071	Monkey Kidney 3196	1745 Clone from tumor produced in animal by High line 3074	2050 Clone from tumor produced in animal by High line 3069	1745 Clone 3423	Human Liver (Epith-Like) 3492
Dehydrocorticosterone (Compound S)	+	?	?	+	+	+	0
Deoxycorticosterone	+	0	0	0	0	0	0
11-Hydroxycorticosterone	+	0	0	0	0	0	0
Androsten	+	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0
11-Dehydro-17Hydrocorti-costerone	+	0	0	0	0	0	0
Cortisol (Compound F)	+	0	0	0	0	0	0

Key
+ - Activity
0 - No activity
- - No Test Run

Significance: These exploratory data collected are of definite value in more accurately classifying cell strains. In addition, such studies as these define more precisely which cells in the mixed cell population in an organ in vivo are responsible for similar activity in the whole organ or tissue slice. Information in this area is one of the most urgent fundamental needs in the advancement of the practical applicability of the long-term tissue culture in cancer research and in other fields.

Proposed course of project: Continuation of this general type of collaboration when appropriate collaborative efforts can be arranged.

Project description: b) Collaborative studies on determination of relative antigenicity of long term strains of human skin and fresh skin cells.

Objective: To determine if species antigenicity is altered after prolonged cultivation in vitro at a rapid rate of proliferation in chemically defined medium both with added serum and in chemically defined medium without any serum. If it is altered by such culture condition, human viruses propagated within the cells might be expect to produce viral tumors in mice or affect experimental animals more readily than virus from original tumor tissue.

Methods employed: Several grams of cells were cultured according to long term large scale tissue culture procedures of the laboratory. They were then injected into mice and various immunological tests were run by Dr. Fink. (See Serial No. 415).

Results: Exploratory results showed that antigenicity of these cells appears to have been reduced by the methods of cultivation in vitro.

Significance: To date no human viruses have been unequivocably isolated from human tumors. Information from this study may aid in making these long term altered cell strains significant tools for human tumor virus research. By their use tumors of human origin might then be studied in experimental animals.

Proposed course: Details indicated in Dr. Fink's report (Serial No. 415). Alterations in both the cells and recipients will be attempted to achieve the objective.

Project description: c) Growth of human skin cells in chemically defined medium in rapidly agitated fluid suspension cultures.

Objective: To define and control factors influencing growth of human skin epithelial cells in agitated fluid suspension cultures. Emphasis has been placed on adaptation of this strain of cells to cultivation in chemically defined medium NCTC 109 with no added serum for the potential in virological research, human tumor research and reparative surgery.

Methods employed: These basically have been previously described (See Project 2, 428 (b). Modifications are thought, however, with this strain, to be responsible for some of the success of growth. Small 250 ml. shaker flasks are lined with Dow silicone (5% concentration in benzene and baked at 250°C for 3 hours) and are inoculated with 25,000,000 cells in 30 ml. of NCTC 109 containing 4000 CPS methylcellulose at 0.45% concentration. The cultures were continuously gassed with 5% CO_2 in air. Cultures were centrifuged in the culture flask prior to fluid renewals and 80% renewals were made. Populations were determined from nuclei counts of aliquots. As soon as a culture reached maximum capacity of fluid allowable in the shaker flask culture they are transferred to increasingly larger size shaker flasks that can still be centrifuged, the largest being a 500 ml. flask.

Results: Cultures grow as single cells and small cell aggregates. In one culture an inoculum population of 25 million cells, under every other day fluid renewals, has increased to 542 million cells within a reasonable interval of 31 days. Cultures from the original culture so established have been maintained for 37 and 43 days and are still under cultivation. Harvesting for daughter cultures is feasible. The results of using the 0.45% 4000 cps concentration of Methocel have been encouraging up to now, although there have been indications in the appearance of the medium that this viscosity may be too heavy to permit maximum proliferation. Future investigations using lower concentrations of methocel are being planned.

Significance: This is the first strain of human cells that has been grown in completely chemically defined medium and also the first that has been grown in such a medium in agitated fluid suspension cultures. The potential for virus assay and virus production as well as possibly reparative surgery should be far reaching and should be investigated by those immediately concerned in these problems.

Proposed course: Effective means of using this cell strain for its full potential significance is open for consultation and collaboration, (See Series No. 415). Continued improvement in the technology of cultivation of this strain of cells will be made, including the point mentioned earlier with respect to modification of concentration and type of methocel for improving the amount and quality of growth.

[illegible]

Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies of Cell Metabolism in vitro.

Principal Investigator: B. B. Westfall.

Other Investigators: L. W. Peppers, V.J. Evans, E.A. Sanford, J. C. Bryant, W.R. Earle, E. L. Schilling.

Cooperating Units: None

Man Years (Calendar year 1959)
Total:
Professionals:
Others:

Patient Days (calendar year 1959)

Project Description:
As before in reports covering the work of this group these investigations are part of the problem study of the cellular origin of cancer. In actuality, several aspects of cell metabolism are under consideration. These are 1) Studies on nucleic acid in long-term strains of tissue cultures; 2) Studies on glycogen content; 3) Studies on the lipid and cholesterol contents; 4) Studies on materials such as amino acids removed from the medium as well as those put into the medium by the cells during growth in the medium; 5) Studies on the keto acid content of used medium; 6) Studies on the enzyme activities, particularly arginase and catalase activity, of long-term cells and of tumors derived from injections of these cells.

Part B included Yes No x

Objective: The first objective is to collect information on these long-term strains. The ultimate objective is to learn if significant differences exist between 1) cells of different origin, 2) cells grown under different culture conditions, and 3) cells of different tumor producing ability.

Methods Employed: Cells and media from long-term cultures in the Tissue Culture Section are used in all these studies. Adaptation and time consuming modification and validation of classic analytical methods are required for practically all procedures employed.

Major Findings: Findings in the nucleic acid study as well as those in the glycogen, the lipid, and the cholesterol studies are being assembled in manuscripts. Strain differences occur in respect to the content of all of these materials. Seven strains of cells, for example, of mouse fibroblast origin had no detectable glycogen. Interestingly enough, however, after several years in vitro, skin epithelium still stores glycogen, and two of the three liver epithelial cell strains studied also store it. Strain 1795 which was derived from a hepatoma arising on injection of tissue culture cells of liver, has lost its capacity to store glycogen; neither does the tumor tissue from mouse injections of these cells any longer have the capacity to store glycogen.

The cholesterol content of the cells in all cell strains studied in chemically defined medium without any serum is decidedly less than when serum is present in the medium.

Nucleic acid studies to date have been confined to mouse cell strains. DNA is higher per nucleus in strain L fibroblasts (NCTC 929 and NCTC 2071) than in cells of fresh mouse tissue, and is also constant, i.e., 17 micrograms per million nuclei 72 hours after fluid change. RNA content per cell is 2 to 3 times as great as the DNA content per nucleus.

Irrespective of the origin of the cell strains, alpha keto acids are formed by all of the cell strains tested; these acids are liberated into the medium at considerable concentrations.

The enzyme activity of many of the cell strains has been examined. The findings on the similarity of keto acid production for all strains are puzzling. The excess may be discharged into the medium because of a ratio use of them at a given time. Similarity among all strains studied in respect to alpha keto acids production points to the considerable similarity in metabolic behavior from strain to strain of cells in at least this one respect.

From the enzyme studies there is the demonstration that cells in vitro can keep some of their characteristic enzyme activity, and in

fact show increases in activity; sometimes they lose all measurable activity. For example the arginase activity of some cells was markedly increased. This indicates that the cells are relatively unstable. In the light of our present state of ignorance of the cause of cancer, such changes in enzyme activity of cells might have significant bearing on the cancer process. Stanfield Rogers has reported nucleic acid from Shope Papilloma virus induced high arginase activity in rabbit skin cells so therefore tumor agents may alter enzyme activity of cells to the degree that a specific alteration is associated with tumor production. It therefore might be possible that the bodies seen in electron microscope sections and judged by Dr. Dalton to be virus-like in the two tumor lines of Sanford with high arginase activity could be associated with this type of phenomenon.

The studies in cell metabolism are considered to have particular significance because the cells studied are from carefully controlled long established cell strains where characteristics and behavior in tissue culture have been observed for long periods of time. Uniformity of treatment of the culture and the availability of much information of the effects on reinjection of cells makes these cell strains of unique value for cell metabolism studies. Considerable emphasis on the arginase activity and catalase activity studies shows that strain differences do occur. In particular all of the liver epithelia are high in arginase. The catalase activity, though low compared with fresh liver, is extremely high compared with fibroblasts. Catalase activity is low in all lines of fibroblast origin. High and low tumor producing lines of fibroblasts (see Sanford 428 (e)) are both higher in arginase than is their parent line.

Significance: Cells can synthesize cholesterol when they are carried in chemically defined medium which does not contain cholesterol or fatty acids.

The finding of low glycogen in the liver strain is unique in that it implies that cells of this tumor strain have lost certain enzyme activities or a single key enzyme concerned with the deposition and mobilization of glycogen.

Cells obviously make protein since they grow. However, in the rapidly growing non-disintegrating culture, they do not throw off of this protein away into the medium. This point is a unique and basic finding which has come out of the use of the completely chemically defined medium. This point has been demonstrated with both the strain L cells and the monkey kidney cells each of which have been grown in protein-free medium.

Proposed course of the work: Great emphasis will be put on the continuation of the studies concerned with materials removed from the medium and those put into the medium by the cells. Significant results should be attained from the unique cell strains which have been carried for long periods of time in medium to which no protein has been added. These should be particularly significant in aiding the program on simplication of the completely defined medium as well as suggesting leads in designing defined media for cell strains which so far have not been grown in any such defined media. Such studies will entail the use of tagged materials using radioactive preparations. This work will be unique because metabolism studies in vitro to date have been confused by not having been done on long term strains cultured in the absence of added protein. Glycine and serine for example increase growth rate of cells; they might therefore be incorporated into the nucleic acids of the cells. Whether or not this is so needs to be determined. Many investigators using serum in the medium have said glycine and serine are not essential in the medium; so this controversial point on the function of these amino acids assumes significance. This and similar studies will be approached through the use of radioactive isotope tracers. Also since cytidine and thymidine are used in the chemically defined medium, studies are now being planned to investigate the possible role of B12 and folic acid in their utilization of these two nucleic acid derivatives by means of tritium tagging.

With an increased number of clone strains of cells now able to grow in chemically defined medium free of added protein, and with increased and increasing simplification of this medium, plans are now being made to continue study along the indicated lines.

NCI
Serial No. 128 (e) - Project 1
1. Laboratory of Biology,
2. Tissue Culture Section,
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar year 1959

Part A

Project Title: Vitamin requirements of a strain of mouse cells as tested in a protein-free chemically defined medium.

Principal Investigator: K.K. Sanford.

Other Investigators: L.T. Dupree, A. B. Covalesky, W.R. Earle.

Cooperating Units: None

Man Years (calendar year 1959):
Total:
Professional:
Other:

Patient Days (calendar year 1959):

Project Description: Our objectives are 1) to simplify protein-free chemically defined medium NCTC 113 by elimination of all vitamins not required by the cells for rapid proliferation. 2) to define precisely the vitamin requirements of these cells under the experimental conditions used. 3) In particular, to evaluate the interrelationships of folic acid, B_{12}, and thymidine for cell proliferation.

Methods employed: Cells of a strain of mouse fibroblasts, NCTC 3071 were grown for 2 months on media lacking each of the 13 vitamins. Strain 3071 is strain 2071 adapted to growth in chemically-defined serum-free medium. After this period, which we found necessary for vitamin depletion, the growth response of the cells to the added vitamin was quantitatively measured. A new medium containing the requisite vitamins is being evaluated and compared for growth stimulation with the original medium.

Major findings: Quantitative studies have been completed on vitamins A,D,K, pyridoxal, pyridoxine, niacin, niacinamide, riboflavin, B_{12}, acid pantothenate, choline chloride, folic acid, thiamin, E, p-amino benzoic, ascorbic acid, and inositol. Experiments are still in progress on biotin. Further evaluation of B_{12} requirements in the absence and presence of folic acid and thymidine is also in progress. Vitamins, which in this medium, are required for cell survival have been identified as panto-thenate, choline chloride, niacinamide, and riboflavin. Vitamins not apparently required for cell survival but which stimulate increased cell proliferation rates are folic, pyridoxal or pyridoxine, and possibly biotin. One substrain appears not to require pyridoxal at all, but confirmatory tests comparing this variant with the original strain are in progress. All of these vitamins have been assayed in the absence of coenzymes. The further investigation of the possible importance of B_{12} will be carried out with chemically defined protein-free medium NCTC 117 containing only two of the four nucleic acid derivatives, deoxycytidine and thymidine, required by these cells in this medium as shown by Evans. With this new medium, the interrelationship of these components for cell growth may be evaluated.

Significance: Studies by Chang and others have shown that dialyzed serum proteins may be contaminated with many different vitamins. For this reason a system such as the one developed in this laboratory, free of added proteins and also antibiotics which could favor intracellular bacterial growth, is unique for accurate studies of vitamin requirements and interrelationships. These studies are significant as a step in simplifying medium NCTC 113 for further comparative studies on nutritional requirements of cell strains. With such a completely defined medium, reduced to only those components the cells actually require, the role of each of the components in normal cell metabolism can then be examined under rigidly controlled conditions.

NCI
Serial No. 428 (e) - Project 2
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project 2, Title: Sarcomatous transformation and persistence of the mammary tumor agent in long-term cultures of mouse mammary carcinoma.

Principal Investigator: K.K. Sanford.

Other Investigators: L.T. Dupree, A. B. Covalesky and W.R. Earle.

Cooperating Units: K.B. Anderwent, Laboratory of Biology, T. Dunn, Laboratory of Pathology.

Man Years (calendar year 1959):
Total:
Professional:
Other:

Patient Days (calendar year 1959):

Project Description
Objective: This project is divided into three parts with three different objectives as follows: Part 1. To determine whether the mammary tumor agent persists in long-term cell strains cultured from spontaneous mammary carcinomas of strain C3H mice. Part 2. To examine the influence of two types of culture media on maintenance of cell differentiation in long-term culture. Part 3. To explain the sarcomatous transformation which occurs frequently in these cell strains.

Part B included Yes No x

Methods employed: Long-term cell strains were established in culture from 9 primary tumors taken from strain C3H female mice with the agent and from 9 transplanted tumors carried for two or more generations in hybrid mice to replace the C3H stroma with stroma from the hybrid host. Such transplanted tumors when explanted to culture would contain the tumor cells and hybrid stromal cells. By suitable transplantation experiments, and by use of single cell clones, the identity of these cell types could be established.

Major findings: The mammary tumor agent appears to persist in certain of these cell strains for periods of over a year. However, no activity of the agent could be demonstrated in cultures that had undergone a sarcomatous change. Differentiation of mammary carcinoma cells was maintained in certain cell strains cultured in serum-supplemented chemically defined medium but not in medium containing serum and chick embryo extract. Experimental evidence indicates that the sarcomatous change in the cell populations may result from either of two entirely different phenomena: 1. Malignant transformation of the stromal cells in culture and 2. Morphologic alteration of the malignant cells of epithelial origin so as to resemble cells of mesenchymal origin.

Cultures of two of these strains have been examined by Levan and Neu who have prepared manuscript on the chromosomes of the cells.

Significance: A problem of practical as well as theoretical interest in studies of the mammary tumor agent is whether the agent continues to propagate in long-term cultures of the tumor. The results of this study are significant in demonstrating activity in certain of the cell strains for periods of over a year. The role of culture media in maintaining cell differentiation in rapidly growing populations of cells has considerable bearing on cell transformation studies.

The frequent reports of sarcomatous transformation in mammary carcinomas transplanted in vivo or in vitro have resulted in considerable controversy as to whether this is a conversion of the carcinoma cell or the result of a malignant change in the stroma. The results of these experiments showed that both processes occurred in culture.

Proposed course of project: A manuscript in preparation will be completed. Certain of these strains will be continued for electron microscope studies by Dr. Dalton.

NCI
Serial No. 428 (e) - Project 3
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project 3, Title: Cell transformations in vitro.

Principal Investigator: K.K. Sanford.

Other Investigators: L.T. Dupree, A.B. Cavalesky, B.B. Westfall, and W.R. Earle.

Cooperating Units: None

Man Years (calendar year 1959):
Total:
Professional:
Other:

Patient Days (calendar year 1959):

Project Description:
Objective: This project consists of a number of separate studies which will be considered under the above heading.

Study 1. Procedure for the growth of single isolated tissue cells. Efforts have been made to simplify and improve the cloning technique and to prepare suspensions of single cells from freshly isolated tissues. An improved cloning procedure has been developed. This study is significant because a procedure for growth of single isolated cells is requisite for studies on cell transformations in vitro.

Study 2. Alterations within a clone of mouse tumor cells. A clonal analysis of a low arginase line of a clone of mouse tumor cells has shown hereditary differences in arginase activity within this line. Differences in enzyme activity between cells of a clone reveal additional hereditary changes affecting enzyme synthesis or activation

Part B included Yes x No

during culture. A manuscript on this study is almost ready for publication.

Study 3. A clonal study of mouse pulmonary carcinoma. A single-cell clone of pulmonary carcinoma is being studied for evidence of sarcomatous change.

Proposed course of project: These studies will be completed. Further studies on the malignant transformation of normal tissue cells in vitro will be pursued. An experiment is being planned to test the role of anaerobiosis as a factor in this change.

Part B Honors, Awards and Publications:

Publications other than abstracts:

Sanford, K.K., Merwin, R.M., Hobbs, G.L., Young, J.M. and Earle, W.R. : Clonal analysis of variant cell lines transformed to malignant cells in tissue culture. J. Nat. Cancer Inst. 23: 1959.

Sanford, K. K., Merwin, R. M., Hobbs, G. L., and Earle, W.R.: Influence of animal passage on a line of tissue culture cells. J. Nat. Cancer Inst. 23: 1959.

Woods, M. W., Sanford, K.K., Burke, D., and Earle, W.R.: Glycolytic properties of high and low sarcoma-producing lines and clones of mouse tissue-culture cells. J. Nat. Cancer Inst. 23: 1959.

Sanford, K.K., Westfall, B.B., Woods, M.W., and Earle, W.R.: Metabolic variations within a clone of mouse fibroblasts transformed to neoplastic cells in vitro. Acta Unio Intern. Contra Cancrum. In press.

Honors relating to this project: Invitation to present a paper to the Washington Pathologists Association entitled "Production of malignancy in tissue cultures of single-cell origin". A.F.I.P Nov. 10, 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project 4, Title: Some effects of polyoma infection on cells in long-term tissue culture.

Principal Investigator: K.K. Sanford

Other Investigators: A.B. Cavalesky, L.T. Dupree, W.R. Earle.

Cooperating Units: A.J. Dalton, Laboratory of Biology,
R.C. MacCardle, Laboratory of Pathology.

Man Years (calendar year 1959):
Total:
Professional:
Other:

Patient Days (calendar year 1959)

Project Description:
Objectives: Our objectives are to determine 1) comparative effects of polyoma infection on 6 cell strains in vitro, 2) evidence for and persistence of virus in 3 cell strains cultured from parotid tumors, 3) some effects of polyoma on a clone of cells from mouse salivary gland tissue.

Methods employed: Cells have been infected by filtered extracts of parotid tumors. Assays for virus activity have been made into Swiss mice less than 24 hours of age. Photographic evidence as well as quantitative growth responses of cells after infection have been made on replicate cultures. Both living cells and fixed and stain preparations have been examined. Assays for malignant potentialities of infected cells have been made into mice.

Part B included Yes No x

Preparations have been given to Dr. Dalton for electron microscope studies and to Dr. MacCardle for examination of golgi and other cell details.

Major findings: Six long-term strains have been compared with respect to maintenance of viral activity and cytopathogenic effect of polyoma infection. Of these, two strains of adult C3H fibroblasts and one strain of chinese hamster tissue cells show no conspicuous effects of infection. Three strains, a clone of mammary carcinoma, a clone of salivary gland(both parotid and submaxillary) cells from normal tissue, and a strain of C3H embryonic cells are susceptible. Effects of infection of the clone from normal parotid and submaxillary tissue are being investigated. Strains cultured from parotid tumors have been studied with respect to activity of virus, cell morphology, transplantability, especially for comparison with the treated normal cells. Many assays have been made, but experiments are still in progress, so results cannot be summarized at this time.

Significance: This project may have significance from two points of view. A clone of cells has been established that may prove one of the most satisfactory for plaque techniques. Cells are susceptible and form a continuous epithelial monolayer on the flask floor. Secondly, possible neoplastic effects of the virus on these cells in vitro, if present, would provide a useful tool for controlled study of carcinogenesis in vitro.

Proposed plan: This project will be continued in an effort to obtain the immediate objectives listed.

No. 428 (f)
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies in tissue culture of the malignant cell and of the normal cell from which it arose. (This is a general title for all work in this Section).

Principal Investigator: W.R. Earle.

Other Investigators: See 428 (a) through 428 (e) preceding.

Cooperating Units: Listed under parts (a) through (f).

Man Years (calendar year 1959):
Total: 19
Professional: 8½
Other: 10½

Patient Days (calendar year 1959):
None

Project Description: The above project may be considered as the general title for all work in the Section. Under the present subhead 428 (f) will be outlined a number of activities not included within 428 (a) through 428 (e).

1. Constant temperature Room 324 has been calibrated tentatively to a satisfactory degree of accuracy and reliability. The room has already been placed in use (September 1959), but only for a small part of the cultures, until sufficient time has passed and enough extreme cold weather seasonal changes have occurred to thoroughly establish the long term reliability of the temperature control.

Part B included Yes x No

The room is already allowing considerable increase in work efficiency, and this will be substantially further increased when "slot" panels for reaching cultures from outside the room have been completed. Two incubators have been discarded and others will follow at a later date.

2. Work and plans for work with the comparative motion picture camera have been described under 428 (a). Equipment is on hand and facilities are being installed for more precise and rapid development of film. Full activation of this development equipment is being accomplished as other equipment is relocated and rearranged to make full use of constant temperature Room 324, Room 322, and Dark Room 331, containing the new film developing machine.

3. More accurate methods for controlling gas tension within cultures are being worked out in an attempt to insure better aerobic conditions within cultures. In collaboration with the Instrument Design Section, Research Services, an apparatus for electronically controlling the gas flow through the gas phase of agitated fluid suspension cultures has been devised and put in use (See 428 (b). Special attention is also being given to alterations in culture design so as to insure more continuous and controlled aerobiosis and/ or anaerobiosis as may be needed to study the influence of these two major factors on cell growth.

4. More precise and reliable methods of media sterilization are now under consideration and study.

5. The check list of publications from the Tissue Culture Section has been brought up-to-date, February 1959, and is now available in limited number for distribution.

6. Better equipment for washing pipettes (use in large number) is now on hand and is being tested.

7. Close collaboration is being maintained with designers of Corning Glass Works in development of a better design of all glass water distillation equipment. One trial unit is now on hand for testing.

8. A patent covering principles and methods of growth of fixed animal cells in deep agitated fluid suspension cultures is still in process (assigned to United States Government). A patent interference by one of the pharmaceutical companies was resolved in favor of Earle et al, except insofar as the methods may be applied to virological research. This exception is unsettled to date.

Patent (assigned to U.S.Government) on the design of the "P" series of culture flasks has been issued; patent covering radical innovations in fabrication is in process pending disposition of an interference action by the Corning Glass Works.

9. The consultancy of W.R. Earle and V. J. Evans with the Tissue Bank, National Naval Medical Center, has been continued. They are in continued intermittent consultation and collaboration, including 1) methodology of freezing and preservation of cells, particularly bone marrow cells, 2) use of the microcinematograph, and 3) adaptation of human skin epithelium. All of these subjects actually fit in very closely with lines of research in the Tissue Culture Section, N.C.I.

10. During the last year we continued to have an extremely large number of visitors, many of whom have been from foreign laboratories or who have come through to evaluate the practicability of setting up tissue culture work in foreign countries. Still another group has been primarily interested in our methods which have maintained culture strains over many years free from demonstrable pleuropneumonia-like infections. Practically all visitors have been primarily interested in tissue culture and its applicability in various lines of work. Many have had specific technical inquiries on which we could advise them with respect to difficulties encountered. Others have wanted to see the general design and operation of the procedures and equipment of the section. Some have asked authorization to remain and work here for an extended time in order to obtain training. While we have tried to assist such workers, lack of space and equipment facilities have forced us to limit the time allowed to what a worker could observe in a limited number of hours. In each instance we try to evaluate the needs of each individual visitor or group. There is no doubt, however, that this handling of visiting professional personnel is extremely time consuming. Further, the time taken up is that of the professional staff because very few visitors are of a type whose desire for information can be met by untrained or subprofessional personnel.

11. Insofar as appears to us practical we try to furnish parent cultures to allow other workers and laboratories to start cell strains in their own organization. No cell strains are furnished which are commercially available, or available from other sources, in satisfactory condition. Cells strains furnished by Tissue Culture Section, NCI, under conditions previously approved by the Director of the National Cancer Institute.

12. The Zeiss inverted (Planktem) microscope is at present considered by far the best microscope for tissue culture since it was first introduced into this field by work and publications of the Tissue Culture Section, Laboratory of Biology, N.C.I. However, this instrument was not originally designed for tissue culture work. In recent conferences in this Section, held with the Chief Microscope Optical and Mechanical Designers of the Zeiss Company, extensive discussions were carried on to redesign this microscope particularly for tissue culture work, incorporating into it various changes which have been made in this instrument in the Tissue Culture Section, Laboratory of Biology, N.C.I. or were suggested by its personnel.

Part B Honors, Awards, and Publications.

Publications Other than Abstracts from this project:

1) See reports 428 (a) through 428 (e).

2) Earle, W.R.: Long-term cultivation of animal tissue cells in large cultures. Fedn. Proc. 17(4) : 967-974, 1958. Presented by invitation April 1958, but published too late to be included in 1958 annual report. See 428 (c).

Honors and Awards relating to this project:

Continued as consultant to Tissue Bank, U.S. Naval Medical School, National Naval Medical Center.

Continued consultation requested by Eli Lilly Research Institute on various technical points in tissue culture. This consultative arrangement is considered of substantial value in advancement of the work of the Tissue Culture Section, National Cancer Institute.

Served upon invitation as Moderator of Conference on Cell Culture, January 13, 1959 at the National Academy of Sciences under the Auspices of the Institute of Laboratory Animal Resources, National Research Council.

Served upon invitation in three conferences during 1959 on "Characterization, Preservation and Supply of Tissue Culture Cell Lines", sponsored by Virus and Cancer Study Section Panel, Research Grants Branch, National Cancer Institute.

Invited to give a series of lectures at the Institute of Cell Physiology, University of Nebraska, Spring 1959. This invitation was held over at my request until Spring 1960 because of pressure of work on the constant temperature room and other equipment.

Presented a paper on invitation, "Long-term Large Scale Tissue Culture", before the Cosmetics Chemists Society, New York, May 7, 1959.

Addressed upon invitation the High School Teachers of Roanoke, Va., on "Long-term Large Scale Tissue Culture in Cancer Research", April 1959.

Addressed upon invitation the Instrument Design Section, Division of Research Services Seminar, NIH, on Tissue Culture, April 1959.

Invited to address the Instrument Design Section, Division of Research Service Seminar, NIH, on the Theory of Tissue Culture December 10, 1959.

Miscellaneous conferences with British Broadcasting Company Television Division on scenario and films for program, October 1959.

Invited to give paper and participate in round table discussion on Carcinogenesis, Berlin, December 1959. Was unable to accept since clearance was not obtainable.

Invited to prepare outline review article for new Scientific American Magazine presenting work of the Tissue Culture Section, Laboratory of Biology, N.C.I. Tentatively accepted invitation pending authorization.

Serial No. NCI-410
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biological and biochemical studies on a murine leukemia virus recovered from Sarcoma 37

Principal Investigator: J. B. Moloney

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2-2/3
Professional: 2/3
Total: 2

Project Description:

Objectives: A comprehensive study of the biology and biochemistry of the leukemia virus, with a view toward an understanding of the nature of the agent and its relation to the neoplastic process.

Methods Employed:

Origin, Source and Extraction of the Virus

Sarcoma 37 which had been carried by M. K. Barrett, of this Institute, for 195 tumor generations in A/JN mice was transplanted to BALB/c mice and carried in this strain for 3 tumor generations before attempting to extract the leukemia virus. It is of interest to note that after 50 passages in mice (BALB/c) the sarcoma still yields the leukemia virus. Similarly leukemia originally induced by cell-free extracts of Sarcoma 37 is readily transplantable within mouse strain and these whole cell induced leukemias (developing in 100 percent of the recipients in 10-14 days following I.P. inoculation) serve as an excellent source of the virus. For additional virus sources see below under tumor variants.

Extraction of the Virus from Source Tissues

Two procedures have been developed for the recovery of the leukemia virus from source tissues. These are (1) a standardized method of differential centrifugation (alternating high and low speed) and (2) a standardized technique employing bacteriological filtration methods in combination with differential ultracentrifugation, a

technique which insures the preparation of an acellular concentrate. The above procedures yield concentrated cell microsome fractions which contain the biologically active virus material. Such fractions derived from Sarcoma 37 and the virus induced leukemic tissues were employed in biological, physical and biochemical studies, the results of which are described below.

Major Findings:

Biological

1. The recovery of virus from leukemic material is primarily dependent on concentration of the agent in the source tissues. To determine from which of the three most grossly affected tissues (liver, spleen, lymph nodes) the greatest amount of virus could be recovered, pools of these tissues were fractionated separately according to procedure (1) above. As determined by latent periods (time to death with leukemia following virus inoculation) and percentage incidence values it was found that leukemic liver is a relatively poor source of the virus. Leukemic spleens and lymph nodes are essentially comparable, both yielding high concentrations of the virus. Cell-free concentrates (0.5 gm. equivalent per ml.) of leukemic lymph nodes when diluted 10^{-4} produced leukemia in 100 percent of the test animals with a mean latent period of 4.5 months.

2. The host specificity of the leukemia virus, with regard to both age and strain of the recipient, was investigated. Age--BALB/c mice ranging in age from less than 1 through 67 days were inoculated with virus recovered from either Sarcoma 37 or the whole cell induced leukemias (see above). A 100 percent leukemia incidence was obtained in all age groups, however, the latent period was somewhat extended in time in the older mice. Results of an experiment now in progress indicate that mice as old as 5.5 months at time of virus inoculation die with leukemia with latent periods as short as 3.0 months. Strain--To determine the degree of strain specificity of the leukemia virus the following strains of mice, kindly furnished from the inbred colonies of H. B. Andervont, were inoculated: BALB/c (reference), C3H, 129H, A/HE, Swiss (general purpose), I, NIII, DBA/2, and C57BL. The resulting experimental data indicate that the leukemia virus is not strain specific. The incidence of lymphocytic leukemia (essentially 100 percent) and the mean latent periods among the inbred strains tested showed no significant deviation from those of the reference strain. Mice of the C57BL strain showed a relatively low leukemia incidence (28.6 percent). This finding might indicate a degree of resistance of this strain to the leukemia virus. This interesting point is being investigated, with experiments in progress designed to determine the effects of both the concentration of virus in the inoculum and the age of the host at the time of virus inoculation (see also NCI-410(a)).

In an effort to determine the susceptibility of the progeny derived from strains showing a low spontaneous incidence of the disease and to further demonstrate any "dilution effect" of strain specificity which might be apparent by out of strain matings, F$_1$ hybrid mice were inoculated with the virus. These progeny resulted from crosses between the males of the inbred strains listed above and the females of the BALB/c strain. All of the animals of the hybrid groups died of leukemia and the average time to death did not vary significantly from the mean survival time of the inbred BALB/c reference strain. Thus, as indicated, no dilution effect of strain specificity was apparent by the out of strain matings.

3. Selective virus passage: The potency of the virus has been increased through a series of experiments termed "selective virus passage." The procedure was to select the first animals of an experiment that presented macroscopic evidence of leukemia. The hematopoietic organs were extracted, filtered, and the virus concentrated by differential centrifugation. Newborn mice were then inoculated subcutaneously with the cell-free virus concentrate. Using such a procedure and after 8 passages the potency of the virus has been increased as shown by a decrease in the latent periods (time to leukemia induction) from the extreme of 6.4 months to 10-12 weeks for 100 percent of the animals.

4. Pathology of the induced disease: Macroscopic--The disease presents the usual picture of lymphocytic leukemia, similar to that which occurs spontaneously in nature. All the diseased mice exhibit greatly enlarged spleens (10-20 times normal weight) and livers. The peripheral and mesenteric nodes show massive involvement, and the thymus is infiltrated to the extent of covering the anterior portion of the heart.

No neoplasms other than those of the lymphocytic type have been observed grossly or microscopically in any of the test animals.

Microscopic--Dr. Thelma Dunn of the Laboratory of Pathology has reviewed the tissue preparations. The neoplastic cell was found to possess a round or slightly indented nucleus surrounded by a narrow rim of basophilic cytoplasm. Mitotic figures were frequent. The architecture of the lymph nodes was usually destroyed and the neoplastic cells extended beyond the capsule. Infiltration by neoplastic cells in the liver was concentrated around the hepatic vessels. Mice with advanced disease showed infiltration of neoplastic cells in the salivary glands, the kidneys, lungs, and in the meninges.

A similar histologic picture was recognized in all the mice examined whether they received virus or were inoculated with neoplastic cells. No variation was detected in mice of different strains or age groups.

The cardiac blood of leukemic mice presented the following picture: Increased WBC (89,250 mm^3), decreased RBC (7,000,000 mm^3), decreased hemoglobin (10.3 gms./100 ml. blood), and decreased hematocrit (34.6 percent).

5. The production of heterologous antiserum: A preliminary analysis of the data from an experiment now in progress indicates that it is possible to produce leukemia virus antiserum in rabbits. Such a heterologous antiserum contains neutralizing antibodies against the virus. Although it has been found that normal rabbit serum does not inactivate the leukemia agent the specificity of the antiserum and its effective titer must be studied.

6. Routes of inoculation: The virus was found to be effective when inoculated by any one of the following routes: subcutaneous, intravenous, intracerebral, and intraperitoneal. Experiments comparing the effectiveness of these routes are still in progress therefore absolute values are not available at this time.

Of interest is the finding that mothers which had cannibalized their virus-inoculated young, later died of leukemia. The possibility of the oral induction of leukemia by virus concentrates is now under investigation. This possibility should be considered in the housing of animals in quantitative bioassay experiments.

7. The effect of thymectomy or splenectomy on the viral induction of leukemia in adult mice is being studied. Eight week old mice were inoculated with virus 48 hours following the operation. The results to date are as follows:

	No. Positive / No. Inoculated	Incidence percent[1]
Control (untreated)	24/27	88.9
Splenectomized	12/15	80.0
Thymectomized	3/14	21.4

[1]Percent of inoculated mice dead or dying with leukemia.

This experiment is in progress, therefore, no average latent period figures are available.

This striking effect of thymectomy on the viral induction of leukemia in mice is being studied further: (a) effect of splenectomy and thymectomy (same individual), (b) effect of thymectomy at various time intervals following virus inoculation and (c) effect of reimplantation of thymus at various time intervals following thymectomy and virus inoculation.

8. The production of tumor variants: Attempts to develop localized leukemias (lymphosarcomas - localized) which would yield the leukemia virus have resulted in the development of the following tumor variants: (a) a lymphosarcoma which arose in the thymic and inguinal node area of an adult mouse which had received leukemia virus by [illegible]

(b) an ascitic form of tumor variant (a) above, originally produced by the intraperitoneal inoculation of the lymphosarcoma, (c) a localized lymphosarcoma grown subcutaneously, originally induced by the inoculation of virus induced leukemic spleen and (d) a localized lymphosarcoma grown subcutaneously originally induced by the inoculation of a virus induced pool of leukemic lymph nodes, spleens and livers. All of these tumors are maintained in routine whole cell tumor transplant series, with passage every 14 days. In all cases the adult mice bearing the localized growth also develop a generalized lymphocytic leukemia.

It is highly significant that all of the tumor variants regardless of their form (solid or ascitic) yield the leukemia virus. Virus preparations, derived from such tumors, when inoculated into newborn mice induce lymphocytic leukemia with latent periods as short as 7 weeks. Mean response values are not available at this time.

Physical

1. The morphology of the leukemia virus as seen with the electron microscope is completely described in NCI-410(a).

2. Stability: The leukemia virus retains its biological activity (tumor producing activity) when prepared in citrate buffer and stored at temperatures below -50° C. Citrate-virus materials prepared in small aliquots (0.3 ml.) are stable and reproducible (biological activity) when sealed under vacuum and stored at the temperature of frozen CO_2. Such "standard" materials may be used in quantitative biological and biochemical studies.

Similarly the Sarcoma 37, the tumor variants and the virus induced leukemic tissues maintain their capacity as whole cell implants and virus source materials when stored at -50° C.

Of considerable technical importance is the finding that the leukemia virus prepared in citrate buffer will withstand lyophilization without apparent loss of activity as compared to a frozen aliquot of the same virus preparation. Such frozen-dried preparations have been sent to selected cancer investigators in this country and abroad.

Experiments now in progress indicate that the citrate-virus suspensions are stable at 37° C. for at least 90 minutes but are inactivated at 56° C. within a period of 30 minutes.

Proposed course of project: In addition to the studies proposed above the following courses of investigation will be undertaken:

1. Biochemical investigations concerned with a more complete understanding of the nature of the virus. These studies will include the role of proteins, lipids, and nucleic acids in the infectivity and metabolism of the virus.

2. Bio-physical studies with emphasis on the stability of the virus under various physical and chemical environmental conditions, i.e., thermal stability, stability in various salt and buffer media.

3. The effect of total body x-irradiation and/or heat on the latent period of the virus induced leukemia in adult mice will be studied.

4. Attempts will be made to cross the species barrier with emphasis on the viral induction of leukemia in rats and hamsters.

5. Purification and concentration of the virus through the application of improved physical and cytochemical techniques.

6. The development of quantitative procedures for the bioassay of the virus employing response criteria other than "time to death with leukemia."

7. Quantitative time-dose-recovery studies of virus from leukemic tissues with particular emphasis on the recovery of virus from leukemic brain.

8. Antigenicity studies to demonstrate an immunological relationship (if any) to other known leukemia agents.

Part B included. Yes X No

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Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Moloney, J. B.: Biological studies on a lymphoid leukemia virus extracted from Sarcoma 37. I. Origin and introductory investigations. J. Nat. Cancer Inst. 24: (April 1960).

Serial No. NCI-410(a)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Collaborative biological, biochemical and physical studies on a mouse leukemia virus recovered from Sarcoma 37.

Principal Investigator: J. B. Moloney in collaboration with those listed below.

Other Investigators: T. B. Dunn, Laboratory of Pathology, NCI
A. Goldin, Laboratory of Chemical Pharmacology, NCI
M. Rachelgl, Laboratory of Biochemistry, NCI
A. J. Dalton, Laboratory of Biology, NCI-406
E. J. Breyere, Laboratory of Biology, NCI-418
R. A. Manaker, Laboratory of Biology, NCI-415
M. Fink, Laboratory of Biology, NCI-415
N. Ida, Present Address: M. D. Anderson Hospital and Tumor Institute, Houston, Tex.

Man Years: (Calendar year 1959)
Total: 2.3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To study, through the collaboration of other investigators, the biological, biochemical and physical nature of the leukemia virus and its relation to the neoplastic process.

Methods Employed and Major Findings: Many of the studies described below are in the early developmental stage; therefore, only experimental results of a preliminary nature are available at this time.

1. The pathology of the virus induced disease has been described in NCI-410. In collaboration with Dr. Dunn of the Laboratory of Pathology a complete oncogenic study of the induced leukemia has been undertaken. This an attempt is being

made to more completely understand the disease process through the early detection of microscopic lesions. At predetermined intervals of time, following virus inoculation, a complete histological examination is made of the organs and tissues of infected mice to determine the pattern (if any) of infection and/or infiltration of the generalized disease. Definitive results from this study are not available at this time due to the short period of observation.

2. The use of this virus for the production of indicator tumors in studies of the chemotherapy of leukemia has two distinct advantages: (a) The induced neoplasm is a host tumor and (b) the induced disease is similar to that which occurs spontaneously in nature. In collaboration with A. Goldin and S. Humphries of the Laboratory of Chemical Pharmacology studies have been undertaken on the chemotherapy of the virus induced leukemia in vivo. These studies have been made possible by the production of sufficient quantities of standardized (known biological activity) virus materials and through an accumulated knowledge of the virus-host relationship. It is possible to produce in one working day sufficient virus to inoculate two million mice all of which will die with leukemia within a known period of time. Further it is possible to determine grossly when a test animal first develops leukemia and to estimate the time to death of the animal (average of 19 days) following first indication of palpable spleen and/or lymph node. Employing such a biological system, selected anti-leukemia drugs are being tested to determine their effectiveness to (a) anti-leukemia virus compounds, (b) anti-virus-induced leukemia compounds, and (c) anti-whole cell induced leukemia compounds. The latter system was originally a virus induced leukemia and carried as a whole cell passage series.

To date no significant increase in survival time of the test animals in relation to the controls has been noted. However, there is an indication that the spleen weight of the treated animals is considerably reduced in relation to that of the untreated animals (see also report by A.Goldin, et al.)

3. Preliminary biochemical studies on mice with advanced virus induced lymphocytic leukemia showed a severe depression of liver catalase activity. This level was below that of animals bearing solid tumors.

Collaborative studies with M. Pachaigl of the Laboratory of Biochemistry are now in progress to determine the time (following virus inoculation) at which liver catalase activity is depressed and to relate this finding with the onset (microscopic) of leukemia and further to correlate this enzymatic depression with the recovery of virus from the affected liver.

4. The morphology of the leukemia virus is being studied in collaboration with A. J. Dalton of the Laboratory of Biology. Electron microscope studies of virus induced leukemia lymph nodes, spleen, liver and thymus glands have been carried out. Similarly studies have been made on crude and partially purified virus concentrates prepared from the above tissues. Virus particles (resembling the A type particle described by Bernhard) have been found in all materials examined.

Within the tissues the particles are found in the intercellular spaces - occasionally within vacuoles in the cytoplasm and infrequently about the inner surface of the plasma membrane. The virus, bound by a double limiting membrane, averages 87 mu in diameter and it possesses a somewhat electron dense nucleoid 48 mu in diameter.

Additional electron microscope studies are in progress to more clearly define the particle, its relationship to the disease process, its nature or morphology in the induced localized lymphosarcomas (see NCI-418) and to quantitatively relate particle number in tissues and prepared virus concentrates to tumor producing activity. Such studies will be of great assistance in virus purification investigations.

5. In report NCI-410 it was noted that the C57BL strain of mice might, to a degree, be resistant to infection by the leukemia virus. Applying this finding and in collaboration with E. J. Dwoyere and M. Burnett (See NCI-418) who have shown that parity influences the host susceptibility to solid tumor implants, a study was undertaken to determine the effect of repeated cross matings of C57BL mice and a susceptible strain of mice on the viral induction of leukemia. These immunological experiments are still preliminary and no findings are available at this time.

6. The leukemia virus has been successfully maintained *in vitro* for extended periods of time in three tissue culture systems by R. A. Manaker of the Laboratory of Biology. These systems are as follows: (a) Virus induced leukemia spleen cells on Earle's mouse liver cells, (b) Virus induced leukemia spleen cells on normal mouse spleen cells, and (c) Infection of normal mouse spleen cells with concentrated virus extracts. For complete details see NCI-416 by Dr. Manaker. Collaborative efforts have been limited to making available virus preparations and leukemic animals. In addition there were made available mice bearing a localized leukemia (lymphosarcoma) described in NCI-410. This tumor has also been successfully cultured *in vitro* by R. Manaker.

7. In collaboration with M. Fink of the Laboratory of Biology the immunological properties of the leukemia virus are being investigated.

To date the antigenic nature of the agent remains obscure for it has not been possible to demonstrate any hemagglutination or complement fixing antibodies associated with the virus. Fluorescent antibody studies with B. Malmgren, Laboratory of Pathology, are in progress and thus far no positive results are available.

It is apparent that aside from the possible production of neutralising antibodies in a heterologous system the virus is indeed weakly antigenic.

8. In the literature there was reported the case history of a child which developed leukemia, being born to a leukemic mother. In several of our experiments it has been noted that the virus is apparently transmitted vertically to the offspring of leukemic parents. Thus at the level of the F_1 generation a high incidence of leukemia is found in these mice born of parents which had received virus when newborn. To determine the route of transmission of the virus to the young a series of experiments was devised in collaboration with N. Ida. Mice known to be pregnant were inoculated with virus at various stages of gestation. Thus one can determine whether the virus is transmitted via the ovum or the placenta. These experiments are in progress and no definitive results are available at this time.

Part B included Yes No X

Serial No. NCI-415
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Application of immunological techniques to the problem of demonstrating viruses in human and other tumors, either directly or by revealing the presence of specific antibody.

Principal Investigator: Mary A. Fink

Other Investigators:

Cooperating Units:

W. R. Bryan and associates (NCI-420)
J. B. Moloney (NCI-410)
R. A. Malmgren (NCI-527)
Virginia Evans (NCI-428)

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

1. Objectives: The major objective is to reveal, by the use of immunological techniques, viruses which are associated with human tumors either as passenger viruses or as etiological agents of the malignancy. The two modes of attack -- demonstration of the virus with specific antibody, or demonstration of specific antibody with a known virus--can be considered together.

Preliminary to the anticipated work with human material, known animal viruses which produce malignancies have been used as models for the testing and development of methods which might be applicable to the human problem.

2. Methods employed: Using the Rous sarcoma virus of chickens and/or the Moloney leukemia virus of mice, attempts have been made to apply the following techniques:

A. Production of antibodies. Formalin treated and heat treated fractions of infected tissue have been mixed with Freund's adjuvant and injected into the homologous species.

Untreated infected tissue fractions mixed with Freund's adjuvant have been injected into rabbits.

B. Tests for presence of antibody:

a) Complement fixation.
b) Hemagglutination-inhibition.
c) Precipitation--in fluid media; in gels, micro and macro methods, single and double diffusion.
d) Fluorescent antibody techniques--on tissue sections to reveal presence of virus; in gel diffusion to reveal an otherwise invisible precipitate.
e) Hypersensitivity techniques.
1. Immediate-type reactions--in vitro Schultz-Dale technique; in vivo anaphylaxis.
2. Delayed-type reactions--skin tests of tuberculin type.

3. Major findings:

A. Production of antibodies. Groups of chickens were successfully immunized against a challenge injection of Rous sarcoma virus by the previous administration of formalin-treated or heated virus mixed with Freund's adjuvant.

Antisera were prepared in rabbits by injecting the extract of leukemic tissue mixed with Freund's adjuvant. The results from the neutralisation test are not complete because of the long incubation period, and until this time we will not know whether or not the antiserum contains antiviral antibodies.

BALB/c mice are being injected with formalin-treated or heat treated leukemia virus.

B. Attempts to demonstrate viral antibodies or antigens:

1. Rous sarcoma virus. Unsuccessful attempts were made to demonstrate antibody to Rous sarcoma virus in the serum of chickens which had regressed several tumors induced by the virus, and whose serum contained a high titre of neutralising antibody. The techniques of complement fixation and precipitation were tried first. Hemagglutination-inhibition studies could not be made because the virus did not agglutinate the erythrocytes of any species tested under various experimental conditions.

The most hopeful lead was with studies employing a fluorescein-tagged antibody in gel-diffusion studies; it is believed that a specific antigen-antibody aggregate not demonstrable by other techniques might be revealed by this method.

In cooperation with Dr. R.A. Malmgren, the fluorescent-antibody technique was successfully applied to show the presence of specifically reacting Rous sarcoma virus in chicken tumors.

2. Moloney leukemia virus. Complement-fixation tests revealed the presence of antibody against mouse antigens, but not against viral antigens. Microsome fractions from either normal or leukemia tissue reacted equally well with rabbit antisera against normal or leukemic tissues. These findings persisted after treatment of the antigenic preparations with Genetron.

Attempts were made to demonstrate a viral hemagglutinin, as a preliminary step to the demonstration of antibody through its role of inhibiting hemagglutination. Erythrocytes of man, chicken, rabbit, guinea pig, sheep and mouse have been tried. Microsome preparations of infected and non-infected tissues have been used fresh and frozen, after treatment with heat, formalin or trypsin. Several times it was observed that hemagglutination did indeed occur, but invariably with the normal tissue to the same extent as with the leukemic tissue.

A lytic phenomenon has been observed repeatedly when the leukemic tissue, but not the normal, was added to guinea pig erythrocytes. We were unable to repeat this with a more highly refined virus preparation the one time this preparation was available. The lytic effect was inhibited by normal rabbit serum as well as the antisera against either the normal or leukemic tissue.

Tests for hypersensitivity to the virus are being done in mice immunized with the killed virus mixed with Freund's adjuvant and subsequently boosted with live virus. These tests include the highly sensitive anaphylactic tests, and a skin test for delayed-type hypersensitivity. Techniques for the demonstration of delayed-type hypersensitivity have been successfully set up using a soluble protein antigen.

In collaboration with Dr. R. A. Malmgren fluorescent antibody studies are being done.

4. Significance to bio-medical research and the program of the Institute.

One of the major objectives of cancer research at the present time is that of determining whether viruses are involved in the causation of human cancer. Since most of the known tumor viruses are species specific, work with human tumor viruses will undoubtedly require more knowledge involving immunological factors as well as the development of experimental methods which take such factors into consideration. The work in progress, and that projected are directed toward both of these ends.

5. Proposed course of project:

Continuation of studies with Moloney leukemia virus until an adequate immunological test system has been devised, or ruled out as a practical possibility.

Application of any findings to the human tumor virus problem as the materials become available.

Attempts to cross species barrier of infectivity in viruses as applied to the establishment of human tumor viruses in mice. This will constitute the major part of the research project during the coming year.

Plan of attempts to cross species barrier of infectivity in viruses as applied to the establishment of human tumor viruses in mice.

A. Rationale: (1) Virus contained in human cell does not infect mice because of immune reaction against foreign species, which destroys medium for propagation of the virus; and/or (2) outer coat of viral particle bears human antigens and an immune response against these prevents viral multiplication. In either case, a breakdown of the host's response to foreign species should provide an environment conducive to viral multiplication.

If one could break down the immune reaction against the human antigens so that human tissue would survive, virus could propagate and perhaps become adapted to the new host.

B. General experimental approach: Break down immunological reaction between the host and the injected virus-containing tissue by:

1. Alteration of the host: X-ray
   Cortisone
   Creation of hypothyroid state
   Creation of deficiency of vitamins known to influence antibody production, e.g., pantothenic acid and pyridoxine
   Develop tolerance by injection of human (and in addition, viral-infected human) tissue in utero or at birth.
   Desensitize by frequent injections of tiny amounts of foreign tissue.

2. Alteration of the inoculum:
   Rid the inoculum of as much of the host material as possible.

   Grow cells in tissue culture, both in serum-containing medium, and in chemically defined medium. If the

growth in tissue culture, this approach might be expected to reveal presence of virus more certainly than inoculation of fresh tissue.

Attempt to reduce species antigenecity by prior freezing of inoculum, and by lyophilization, so long as these procedures do not adversely affect the infectivity of the virus. (There is evidence from human homografting that these physical procedures do reduce the antigenicity).

C. Specific experimental approach:

1. It is proposed that all of the methods outlined under Alteration of the host be attempted first with the Rous sarcoma virus to which system. This choice is made because of the large body of background information on this tumor virus. Actual experimental work is awaiting the completion of an animal room.

2. In collaborative studies with Dr. Virginia Evans, Tissue Culture Section, results from a small number of animals inoculated with human cells which have been in tissue culture give a strong indication that the antigenicity of these cells has been reduced for the mouse. These experiments will be continued until a valid conclusion can be made regarding the efficacy of the procedure in reducing the antigenicity [illegible] with species specificity.

Part B included Yes X No

NIH-NIH
Individual Project Report
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Part B. Honors, Awards, and Publications.

Malmgren, R. A., Fink, M. A., and Mills, W.: Demonstration of the intracellular location of Rous sarcoma virus antigen by fluorescein labeled antisera. J. Nat. Cancer Inst., accepted for publication.

By invitation from Tissue Bank, National Naval Medical Center, delivered a series of six evening lectures on Basic Immunology to the staff of the National Naval Medical Center.

Serial No. NCI-416
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Viral Therapy of Tumors. I. Adaptive Measures for Increased Oncolysis in Cases of Advanced Carcinoma of the Cervix

Principal Investigator: Robert A. Manaker

Other Investigators: Robert B. Couch, Edward D. McLaughlin

Cooperating Units: This project complements the clinical aspects of viral therapy of carcinoma of the cervix as conducted under the direction of Dr. Robert R. Smith, Chief, Surgery, NCI, and extends the work initiated by Drs. Robert J. Huebner and Wallace P. Rowe, Laboratory of Infectious Diseases, NIAID, 9-752-C.

Man Years (calendar year 1959):
Total: 1/3
Professional: 0
Other: 1/3

Project Description:

Objectives: To continue the investigations in progress to determine the extent to which the oncolytic properties of Coxsackie B-3 virus could be enhanced by tumor to tumor passage.

Methods: Tumor to tumor passage of Coxsackie B-3 virus was conducted in patients with carcinoma of the cervix. An arbitrary goal of 10 serial passages was set. The virus inocula used were prepared in HeLa cell cultures infected with a late positive specimen obtained from the preceding patient. The preparation of inocula in Maitland type cultures of virus infected tumor tissue was unsatisfactory.

Major Findings: Fifteen patients with cervical carcinomas were treated with Coxsackie B-3 virus. In the course of this treatment, four additional passages were made. A total of ten tumor to tumor passages of virus have now been made. The tenth passage has been inoculated into two patients. No results indicative of increased oncolytic activity solely due to passage in cervical tumors were obtained. The clinical findings will be reported by Surgery, NCI.

Significance: This study of the oncolytic activity of Coxsackie B-3 virus in carcinoma of the cervix provides a model for evaluation of adaptive techniques in viral therapy of human tumors.

Proposed course: This program is of necessity geared to the availability of suitable cases for trial. For this reason, a limit of ten serial virus passages in cervical tumors was set to provide data for evaluation of the adaptive measures used. That limit has now been reached. Final decision of the course of this project is expected in the near future.

Part B included Yes No X

Serial No. NCI-416(a)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Application of Tissue Culture to the Study of Tumor Viruses. I. Effect of Mouse Tissue Culture Material Previously Treated with Human Leukemic Brain Extracts on Tumor Incidence in Swiss mice

Principal Investigator: Robert A. Manaker

Other Investigators: None

Cooperating Units: Parallels and extends the joint project of Dr. Sarah E. Stewart (NCI-433) and Dr. Bernice Eddy, DBS, LVP; Pathology by Dr. Mearl Stanton, IP, NCI.

Man Years (calendar year 1959):
Total: 1-2/3
Professional: 0
Others: 1-2/3

Project Description:

Objectives: Investigation, in Swiss mice, of the tumor-producing potential of fluids from mouse embryo tissue cultures previously inoculated with extracts of human leukemic brain tissue.

Methods: Two techniques were applied. (1) Following the basic procedure used by Drs. Sarah E. Stewart and Bernice Eddy, cultures of whole Swiss mouse embryos were maintained in medium 199 supplemented with calf serum. These cultures were inoculated with extracts of human leukemic brain tissue. Serial blind passage of fluids was made at bi-weekly intervals. Each passage was terminated after 4 weeks of incubation. Newborn Swiss mice were inoculated with culture material after the second, third, and fourth week of incubation. Control mice received untreated tissue culture material. (2) All human brain tissue specimens were retested in Swiss mouse embryo cultures maintained in medium 199 supplemented with rabbit serum. Serial blind passage of fluids to fresh cultures was made weekly and twice weekly. Ground cells and supernatant fluids were used as inocula for passage.

All mice were identified individually and examined periodically. At death, mice were examined grossly for tumors, and specimens were removed for pathological examination.

Major findings: Nine specimens of brain tissue from cases of leukemia and 5 specimens of brain tissue from non-leukemic sources were tested. The results obtained with 1314 inoculated control mice, 287 uninoculated mother mice, and 971 test mice showed no significant difference in the types of tumors which occurred in the three groups 1.5 to 2 years after inoculation. Nor were significant differences observed in the number of tumors found in the test group when compared with the number found in the control group. Most tumors were found in old animals. Lung tumors, mammary gland tumors, and leukemia were the malignancies most frequently encountered.

Significance: This project provided a test of the suitability of mouse tissue culture as a means for isolating viruses from leukemic brain and of the mouse as a test animal for tumor induction by such isolates.

Proposed course: The project has been terminated.

Part B included: Yes No X

Serial No. NCI-416(b)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Application of Tissue Culture to the Study of Tumor Viruses. II. Viruses Associated with Human Tumors

Principal Investigators: Robert A. Manaker, Robert B. Couch

Other Investigators: Edward D. McLaughlin

Cooperating Units: Dr. Robert R. Smith, Chief, Surgery, NCI

Man Years (calendar year 1959):
Total: 1-1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: Determination, by application of tissue culture techniques, of the extent to which viruses may be demonstrated in association with a variety of human tumors and to investigate the nature of such agents.

Methods: Two techniques were employed to disclose the presence of viruses in surgically-removed tumors: (1) Culture and observation of tumor tissue for visible evidence of virus activity. (2) Inoculation of a variety of cell lines with minced viable tumor tissue, observation for a month or more, and blind passage to fresh cultures for an equivalent period of observation. The first technique was carried on by the Surgery Branch, NCI. Fluids and cell material from this source were further investigated by the second technique. The latter was carried on by the Laboratory of Biology, Virus Oncology Section, NCI, and is herein reported.

The available cell lines included a normal human "skin" line, a "bone" line which originated from human epiphyseal tissue, and a monkey kidney line. These were obtained from Dr. Wilton Earle as stock cultures. Human embryo skin-muscle and human intestine cultures were obtained from Microbiological Associates, Bethesda, Md.

Major Findings: A total of 15 surgically-excised tumors were introduced into the available cell lines as described. In addition, 27 specimens of tissue cultured tumor material received from Dr. R. Couch of the Surgery Branch, NCI, were similarly processed. No evidence of virus activity has been observed to date.

Significance: The association of viruses with tumors in animals makes mandatory the development of methods for determination of the extent to which viruses may be found in human tumor tissue.

Proposed course: This program will be further developed during the following year.

Part B included: Yes No X

Serial No. NCI-416(c)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The application of tissue culture to the study of tumor viruses. III. Maintenance of a leukemia virus of mice in vitro

Principal Investigator: Robert A. Manaker

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2-2/3
Professional: 2/3
Others: 2

Project Description:

Objectives: To determine the possibility of routine in vitro maintenance of the mouse leukemia virus isolated by Dr. J. B. Molomby, NCI.

Methods: The experimental patterns comprised: (1) Tests for the persistence of the leukemia agent in cultures of mixed thymus, spleen, and lymph nodes obtained from leukemic animals. (2) Attempts to adapt the leukemia agent to an established mouse liver cell (ML) line (Earle) with inocula of leukemic tissues. (3) Determination of the possibility of in vitro infection of normal mouse thymus or spleen tissue. (4) Investigation of the suitability of mouse spleen cultures for the maintenance of the leukemia agent in serial passage. (5) Characterization of passenger viruses which might confuse the interpretation of the results obtained in cultures containing the leukemia agent.

In the absence of definitive cytopathogenic activity by the leukemia agent under the conditions employed, all tests for the presence of virus were made in mice. Results were available 4 to 6 months later.

Major findings: 1. The persistence of the leukemia agent in the fluids of primary cultures of tissues from leukemic animals for extended periods was demonstrated. Alternate in vivo - in vitro passages were also made. In one experiment, amoeboid cells, which had their origin in the liver or in mixtures of thymus, spleen and lymph nodes of leukemic mice, were cultured for a period of 30 days.

Samples of fluids taken at the 10th and the 35nd day of culture were divided into 2 parts. One part was filtered through a bacteria-tight filter to remove cell material. Both filtered and unfiltered samples were injected into litters of newborn mice. A total of 35 mice were thus inoculated. Within 3 to 6 months, all but one died of leukemia. No evidence of the presence of malignant cells in the cultures was noted. No difference in latent period was noted for those animals that received only fluids when compared to those animals that were inoculated with fluids containing viable cell material.

To investigate the ability of a lymphoid cell to maintain the virus, a lymphosarcoma which had its origin in an animal previously inoculated with active extract of leukemic tissues, was obtained from Dr. John B. Moloney. Cultures of the malignant cell were maintained for 7 weeks. Fluids containing cell material produced tumors in 2.5 weeks when inoculated into mice subcutaneously. Fluids sampled after 4 and 7 weeks of culture were filtered through Seitz filters of 03 porosity to remove cells. Leukemia resulted in 80 percent of animals inoculated neonatally with fluids from 4 week cultures, and in 50 percent of animals inoculated neonatally with fluids from 7 week cultures. Deaths from leukemia occurred between 6 and 9 months after inoculation. Two month old mice also received these fluid inocula. Deaths from leukemia occurred in 7.5 to 9 months in 2 of 6 animals inoculated with the 4 week fluids, and in 2 of 8 animals inoculated with the 7 week fluids. The results suggest that the lymphosarcoma cell supports the virus poorly if at all. The presence of the agent may have been due to a small population of another cell type.

2. An attempt to establish the leukemia virus in Earle's cloned mouse liver cell line was made. Over a period of 2.5 months, 4 additions of spleen and lymph nodes from leukemic mice were made to mouse liver cultures in flasks. Three days after the last addition of tissue, fluids were filtered through a Millipore HA filter and used to inoculate fresh flasks of the mouse liver line. At this time the inoculum was tested for activity by the inoculation of newborn mice. All animals inoculated died with leukemia 3 to 5 months later. Tests were made of fluids from the cultures inoculated with the filtrate beginning with the second day post-infection. Nine tests were made during the next 2 months. None of the animals injected with these materials has become leukemic during 5 to 7 months' observation. Evidently, no adaptation occurred.

The original cultures apparently are supporting cell material from the tissue inocula. Leukemia was produced in animals 2.5 to 5 months after neonatal injection of culture fluids taken 8 weeks, 9.5 weeks, and 14 weeks after these cultures received the last inoculation of leukemic tissue. Fluids in these cultures were routinely changed completely thrice weekly.

3. To determine the possibility of *in vitro* infection of spleen and thymus tissue cultures prepared from normal BALB/c mice, a potent cell-free extract of leukemic mouse tissues was obtained through the courtesy of Dr. John B. Moloney. This extract was used to infect cultures which had been maintained for 17 days *in vitro*. The leukemia agent was present in both cultures 14 days thereafter, as shown in animal tests.

4. Serial passage was attempted in mouse spleen cultures. Three experiments were carried out. Only the first of these has been under way sufficiently long to yield significant results. The experiment consisted of 3 series of cultures. A schedule for passage of one-tenth of the fluids from tubes of each series to fresh tubes each week was adhered to as much as possible. The first series consisted of uninfected control cultures, the second consisted of cultures infected with cell-free extract of leukemic mouse tissues prepared by Dr. John B. Moloney, and the third consisted of cultures infected with 0.01 ml. of viable, minced leukemic spleen, lymph nodes, and thymus of a mouse bearing the 14th transplant passage of leukemic cells. Fluids in cultures were changed completely 3 times each week. Newborn mice were inoculated with fluids and cell material from each passage with the exception of the 9th, 12th, and 13th. The last passage made was the 14th.

No leukemia occurred in the inoculated control mice. No leukemia has been observed beyond the fourth passage of fluids from extract-infected cultures. One of 7 mice that were inoculated with material from this fourth passage culture died of leukemia in 3.25 months; the remainder are still being observed. The leukemia agent in those cultures infected with tissue has been maintained for 11 serial passages. One of 7 mice inoculated with 11th passage culture material is leukemic 2.5 months after neonatal injection of test culture material. Leukemias have occurred in animals used to test the prior passages for presence of the agent. The agent can be maintained *in vitro*, but its behavior under these conditions is not yet clear.

5. Two viruses which produce hepatitis in mice were isolated during these studies. The first isolated was more extensively investigated than the second. A paper reporting the results obtained is being prepared for publication.

The hepatitis virus most extensively studied has been designated MHV-A59. It is stable to ether, a characteristic differentiating it from the second agent isolated. Heating at 56° C. for 30 minutes destroyed infectivity. Infection in the BALB/c mouse induces immunity. Serum from immune animals has a relatively high neutralization titer. These properties differentiate this agent from others that have been previously reported in the literature. Immune mothers transmit resistance to infection to their progeny within hours of birth. The virus has been passaged in tissue cultures of Earle's mouse liver line and in mouse spleen cultures. Cytopathogenic changes are produced in both systems.

Significance: Maintenance of the leukemic agent in vitro makes possible controlled studies and permits investigations not possible in vivo. The identification of a passenger virus (hepatitis) and development of methods for its propagation will permit its elimination through immunization, and thus a more adequate control of the biological studies on leukemia.

Proposed course: This program will be further developed and extended during the next year.

Part B included: Yes No X

Serial No. NCI-420(c)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Rous sarcoma virus.
III. Further studies on the relation of recoverable virus to tumor initiating dose.

Principal Investigator: W. Ray Bryan

Other Investigators: Dorothy Calman, John P. Kvedar

Cooperating Units: Albert J. Dalton and associates (NCI-406)

Man Years (calendar year 1959):
Total: 1-1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: To determine whether there is a quantitative correlation between the incidence and/or population density of viral particles seen in thin section electron micrographs of tumors and: (a) the dose of virus used to initiate the tumors, or (b) the amount of extractable virus determined by bioassay.

General comment: The experimental work associated with this project was completed in October 1959, and the Methods employed are therefore not reiterated (see 1958 report).

Major findings: Analyses of the bioassay data show the same correlation between the amount of virus extractable from tumors and the initiating dose of virus as was previously found, and reported. It is the correlation of these findings with virus-particle counts under the electron microscope that constitutes the chief purpose of this project. The electron micrographic studies are in progress in Dr. Dalton's laboratory, but have not yet been completed. Final evaluations await these results.

Significance: The results are expected to contribute to basic knowledge concerning the mode of action of tumor viruses in bringing about malignant transformation.

Proposed course of project: Completed except for data analyses and writing of report for publication.

Part B Included: Yes No X

Serial No. NCI-420(d)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, MD.

FWS-7112
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Rous sarcoma virus. IV. Investigations on host response to the virus (a) in chickens and (b) in Japanese quail

Principal Investigator: W. Ray Bryan

Other Investigators: P. J. Rauscher, Dorothy Calnan, John P. Kvedar

Cooperating Units: J. A. Reynolds and associates of the Germfree Life Research Center, Tampa, Fla.

Man Years (calendar year 1959):
Total: 2-1/3
Professional: 1/3
Other: 2

Project Description:

Objectives: (a) To determine the ultimate limit to which the concentration of virus in Rous sarcoma tissue can be increased through continued selective serial passages in chickens; (b) To determine the suitability of Japanese quail (Coturnix japonica) as a host for the study of Rous sarcoma virus.

Methods: (a) Past experience has shown that the virus concentration of Rous tumor tissue could be progressively increased by selecting as a source of virus at each passage that tumor which appeared first, and grew most rapidly, in a group of about 40 chickens. Such serial passages are being continued routinely, and will be maintained until such time as an upper limit of virus concentration is reached, and no further increase with passage is detectable.

(b) Exploratory experiments in collaboration with Dr. Reynolds have shown that the Japanese quail is highly susceptible to the Rous sarcoma virus. Further studies are being conducted, including passages in quail similar to those in chickens (see (a)), to determine whether the quail (a very small fowl) can replace chickens in laboratory studies of the virus.

Major findings: (a) At the time of the last report (1958) a total of over 40 passages had been made and the overall potency of tumor tissue extracts had been increased by 1.5 log units, through selective passages. The study is now in the 62nd passage and potency has been further increased by 0.6 log units (4 fold) during the last

year. This represents a total increase of 2.1 log units (over 100 fold) in potency over the materials available before this study was undertaken.

(b) Preliminary studies have shown that the Rous sarcoma virus titrates out as far in quail as in the most susceptible chickens yet investigated. Also, the yields of virus from tumors of quail induced with strong doses are equal to the highest yields obtained from the most susceptible chickens.

Significance: (a) The purpose of attempts to increase virus content of tumor tissue is to permit purification of the virus in quantities adequate for physical, chemical and immunological studies. The potencies already achieved would make it practical at this time to undertake purification studies. However, the task will be easier the higher the virus content of tumor tissue, and attempts toward this end will be postponed until the upper biological limit of virus concentration has been definitely established.

(b) If the results with quail are readily reproducible the quail will constitute a very valuable host for studies on the Rous sarcoma virus. The chief advantage will be in the smaller size of the test animal, which is no larger than a 3 day old chick when full grown. This would permit 5 to 10 times as much investigative work to be done within the same space as is now devoted to chickens.

Future course of project: (a) Selective passages will be continued until an upper limit of virus concentration in tumor tissue has been reached. (b) More extensive experiments will be carried out to test the reproducibility of the preliminary finding with quail and to determine their suitability as test animals for this virus.

Part B included: Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bryan, W. R.: Enhancement of virus yield of Rous sarcomas. Acta Unio. Internat. contre le cancer 15: 764-767, 1959.

Bryan, W. R.: Quantitative biological experimentation in the virus and cancer fields. J. Nat. Cancer Inst. 22: 129-159, 1959.

Honors and Awards relating to this project: None

Serial No. NCI-420(f)
1. Laboratory of Biology
2. Virus Oncology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Search for tumor inducing viruses among mammalian tumors (including human) not now known to have a viral etiology

Principal Investigator: W. Ray Bryan (Director of Sectional participation in broad program)

Other Investigators: M. A. Fink, E. A. Manaker, F. J. Rauscher, Dorothy Calnan, John P. Kveder

Cooperating Units: H. B. Andervont and associates - NCI-400
A. J. Dalton and associates - NCI-406

Man Years (calendar year 1959):
Total: 7.5
Professional: 2.5
Other: 5

Project Description:

Objectives: To determine whether various mammalian tumors that occur spontaneously in laboratory animals and humans are associated with viral etiological agents.

General statement: This project is part of a larger collaborative study and involves four independent investigators and two research assistants of professional grade within the Virus Oncology Section.

The activities of this Section constitute a primary part of the larger collaborative study which involves other Sections of the Laboratory of Biology, and other laboratories of the National Cancer Institute, as well as two Public Health Service Hospitals. Coordination between sections and laboratories is provided by the chief of the Laboratory of Biology. Overall direction and coordination of the joint studies involving PHS hospitals is provided by the Associate Director in Charge of Research, NCI.

Methods employed: Since tumor viruses of laboratory animals are species specific, human tumor viruses, if they exist, may also be expected to be species specific. This constitutes the major obstacle to an all-out attack on the human problem with existing knowledge and techniques, since biological tests for viruses cannot be carried out within the same species.

The most promising approach to the human problem at the present time is that of using human cells grown in tissue culture as biological indicators of virus activity. This approach was therefore started first, and has now been in progress for about one year (Dr. Manaker and associates - see his report, NCI-416).

A second approach involves the use of recent developments in the field of immunology and the injection of laboratory animals in the late foetal or neonatal state for the induction of immunological tolerance toward foreign (human) viral antigens in laboratory animals. Exploratory studies, only, have been carried out thus far, pending the availability of animal rooms now under construction (see report of Dr. M. A. Fink - NCI-415).

The third approach now being pursued involves the inoculation of both filtrates of mammalian tumor tissue and tumor tissue fragments onto the chorioallantoic membranes of both chicken and Japanese quail eggs. Technical procedures are being developed with tumors of laboratory animals not now known to have a viral etiology. Studies involving human cancers will be instigated as soon as the technical procedures have been sufficiently developed to insure a fair chance of success. Until recently these studies were conducted by the head of the Virus Oncology Section, but have now been turned over to Dr. F. J. Rauscher who recently joined the staff.

In addition to the above biological approaches, studies in collaboration with Dr. Dalton and his associates of the Cellular Biology Section involve examination of tumor tissues by electron microscopy for evidence of the presence of virus particles. Toward this end, candidate tumors are extracted and processed by methods that have proved successful in work with known animal tumor viruses, and concentrated fractions which would be expected to contain particles of the size of viruses are derived by ultracentrifugation. The pelletized materials are fixed while in the centrifuge tubes and then processed for thin section electronmicroscopy. Examinations of all strata of the pellets in longitudinal sections provides a basis for the detection of virus or virus-like particles, if they are present (See report of Drs. Dalton and Zeigel).

Finally, a major part of the overall research project involves the similar testing and study of mammalian tumors other than those of humans, but which are not now known to have a viral etiology. Since such materials can be put back into indicator hosts of the same species (various laboratory animals), a means is thus provided for proving the efficacy of the various procedures and approaches outlined above for the detection of tumor viruses.

Major findings: The past year has been spent in the development of methods, recruiting of new personnel, and the remodeling of laboratories and animal rooms. Although actual investigations are already underway, the program is not yet in full operation. No final results are available for reporting at this time.

Projected course: During the calendar year 1960 the following man years will be devoted to this project:

Total:	13.5
Professional:	3.5
Other:	10

Part B included: Yes No X

Serial No. NCI-250
1. Lab. Chemical Pharmacology
2. Office of the Chief
3. Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Planning, Management and Coordination of Investigations, Organized in an Interdisciplinary Team Approach, on Basic Science Aspects of Pharmacology in Cancer.

Principal Investigator: Murray J. Shear.

Other Investigators: Adrian Perrault.

Cooperating Units:

Man Years (calendar year 1959): Patient Days

Total:	11	None
Professional:	2	
Other:	9	

Project Description:

A. General

1. Plan

The strategy has two main facets: a) investigation of factors in the mammalian host which are capable of influencing major phenomena in malignancy — viz., genesis, growth, invasiveness, and metastasis; and b) contributions to principles of methodology, and mode of action, in drug treatment of cancer. Although these two main lines of work would appear to be unrelated, some of the findings will require interdigitation of the immunological and the chemotherapeutic approaches for their exploitation.

The points made in this part of the report last year continue to have relevance.

2. Organization

Re-orientation and reorganization of the Macromolecular Chemistry and of the Biochemical Pharmacology Sections, described in the previous annual report, have now been accomplished.

Dr. A. W. Schrecker is performing ably, with high morale, together with Dr. J. A. R. Mead who plans to stay with us and become a citizen of the United States; they work well and fruitfully together. Dr. M. A. Chirigos joined Dr. Goldin's group during the year and is taking hold in a quite satisfactory fashion. Mr. John Venditti is functioning, to our complete satisfaction, as Assistant Project Officer in the scientific operations under contract arrangement at Microbiological Associates. The work of Dr. Goldin and his staff has increasingly been receiving favorable recognition. So many requests are being made of Dr. Goldin to provide his experience and judgment to affairs of chemotherapy on the broader scene that, in view of his whole-hearted co-operative spirit, there is now the danger of his becoming overloaded.

Dr. P. T. Mora is continuing to mature, as a biological scientist and as a section leader, in a gratifying fashion. The work he and his collaborators have been doing has attracted favorable comment from outstanding research men in his field both here and abroad. The following personnel changes are being made: Dr. D. Vivian's retirement for disability becomes effective at the end of 1959, and his technician is being transferred; Dr. J. Preiss is also being transferred; and Dr. E. Merler is leaving for a university appointment in January 1960. Recruitment for replacements is in progress.

In the Polysaccharide Section also, there is a continual ferment of exciting ideas and findings, which go on despite personnel turnover. In the spring, Dr. R. Skarnes left to work in Prof. Grabar's laboratory

in Paris for a year under an NIH Fellowship and, at the end of June, Dr. F. Rosen returned to Boston under an appointment at the Children's Medical Center of Harvard. The replacements are two young men: Dr. G. Michael who recently received his Ph.D. in Microbiology at Rutgers under Prof. Werner Braun; and Dr. W. R. Keene, who had just completed his internship at the Johns Hopkins Hospital. Both men show signs of promise.

Towards the close of the year, realignment of personnel among the Sections was carried out to depict more realistically the actual supervisory relationships. The major change was the shifting of Mr. Perrault and his subordinates to the Office of the Chief.

B. Specific

The activities of the Laboratory are described in detail in the accompanying reports by the four Section Heads. At this point, a few specific items are selected for mention.

1. Further integration of the laboratory program with the clinic is in progress. Dr. Goldin continues to maintain a close and regular liaison with the Clinical Director and the staff of the Medicine Branch. Steps are being taken to extend to clinical material from the Surgery Branch the findings made, with animal material, in the sections dealing with macromolecular biology and chemistry.

2. Dividends are beginning to be obtained from studies of host factors in the mammal which affect bacterial polysaccharides and bacterial cells. These findings are valuable in themselves but, in addition, the bacterial cell has been employed as a "model" for the mammalian cell. Extension to tumor cells of our newer concepts and materials is yielding positive progress.

3. We are still wrestling with the difficult problem of unambiguous isolation of polysaccharides with endotoxic properties from mammalian material. This developed from the experiments of Dr. E. Merler whose attempts to confirm and to systematize the empirical procedures of Mr. Perrault highlighted the necessity to rule out, rigorously, the role of contaminating bacteria by definitive culturing of samples of the material taken throughout the processing procedures. Mr. Perrault has now succeeded in governing the protracted manipulations, over many weeks, in the open laboratory so that bacterial contamination (as checked, independently, at every stage by Mr. Trapani) is not a factor. The final products obtained in this way, however, have been unexpectedly large in amount, and high in nitrogen content, as well as devoid of endotoxic characteristics. Work is in progress to split off the polysaccharide moiety from the proteins with which it is presumably bound.

4. Requests continue to be received by the Chief for consultation, advice, and help in many different kinds of scientific matters. These appeals come from various parts of the NIH, other Federal Government Departments, universities, scientific organizations, industry, and Embassies of foreign countries. The requests come from this country and abroad, and are made down the line from Directors of Research to recent graduates and students. These demands are met within the limits of feasibility.

5. A good deal of time has had to be given by the Chief to the affairs of the American Association for Cancer Research in discharge of his official responsibilities in that organization as President-Elect, as Chairman of its new Policy Committee, and as its representative in the Washington area.

6. Item 6 in the report for 1958 stated that "International research activities claimed an increasing

share of the time" of the Chief. This was even more the case in 1959. He continued to contribute, with others, in the drawing up of proposals for world-wide cancer research through WHO. He re-organized, as Chairman of the Finance Committee of the International Union Against Cancer, the obtaining of new financial support for the UICC, and continued to serve as Chairman of the Chemotherapy Committee of the Union. In his capacity as an officer of AACR and of UICC, he devoted (by invitation) considerable time to staff work for legislative circles.

Future Course:

The general philosophy of the program of the Laboratory is much the same as described in the report for 1958. Coordination and interdigitation among the various projects in the different sections will continue to be encouraged.

Directing research is relatively easy: a) when instructions are issued by the Boss Man, and followed by a docile staff (i.e., Regimentation); or b) when in a group of creative original men each, separately, follows his own bent, with the Chief merely being their administrative servant (i.e., Laissez-Faire). Giving leadership to talented, strong-willed, temperamental scientists is difficult enough when each works on a completely independent project, but the difficulties are multiplied many-fold when the objective is to maintain their enthusiasm for working toward a common target and with materiel in common. But it is worth the effort!

Discussions have been held by the Chief with several of the staff of the Surgery Branch to the end that this Laboratory might explore possible differences between the blood sera of cancer and non-cancer patients with regard to the newly-uncovered cytocidal factor for tumor cells; also, to determine whether human cells (normal and malignant) behave as do those of mice with respect to capacity to absorb out this factor from normal human blood serum. In the light of the work of the Björklunds in Sweden,

and of Fedoroff in Western Canada, this is on the docket. It only remains to persuade the leading men in the Laboratory to give these a sufficiently high priority.

At first sight the mechanism of carcinogenesis by chemicals might seem to have no obvious relationship to the other lines of work in this Laboratory. However, there are now reasons for suspecting that the host factors presumed to be involved in other neoplastic phenomena under study by us may also play a part in the generation of tumors by carcinogens. Hence we propose to explore this hunch during the coming year.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications (no abstracts included):

(1) Landy, M., Trapani, R-J., and Shear, M. J.: Inactivation of endotoxin by a humoral component. IV. Alteration in the immunological properties of typhoid endotoxin. J. Exper. Med. 110: 731-750 (1959).

(2) Mora, P. T., Young, B. G., and Shear, M. J.: Reduction of Toxicity of Cationic Macromolecules by Complexing with Anionic Derivatives of Synthetic Polyglucoses. Nature, 184: 431-433 (1959).

(3) Mora, P. T., and Shear, M. J.: Reversible Inhibition of Macromolecules (Enzymes, etc.) by Electrostatic Interaction with Derivatives of Synthetic Polysaccharides. Internationale Union für Reine and Angewandte Chemie, Kommission für Macromolecüle, Symposium über Makromolekülle in Wiesbaden. Macromoleculare Naturstoffe und Modelle, Vortrage V C3, Verlag Chemie, GmbH. Weinheim (1959).

Honors and awards pertaining to this project:

1. Elected Vice-President (President-Elect) of American Association for Cancer Research at the Annual Business Meeting, Atlantic City, April 1959.

2. Appointed Chairman of the newly-created Committee on Policy, of the Amer. Assn. Cancer Research.

3. Many people have commented on the inclusion in the lead article in the "Saturday Evening Post" for Oct. 31, 1959 of an interview (and photograph) with the Chief on cancer chemotherapy. It is interesting that they consider this to be "recognition and an honor"!

4. CBS has made a film, with sound track, of the interview of the Chief by Dr. Tom Dooley (of Laos) for broadcast in January or February some evening in the program "CBS Reports . . . . . ".

Serial No. NCI-253
1. Laboratory of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemical and Pharmacological Studies of Host-Tumor Relationships in Tumor Chemotherapy.

Principal Investigator: Dr. A. Goldin

Other Investigators: Dr. A. Schrecker, Dr. M. Chirigos, Dr. J.A.R. Mead, S. Humphreys, J. Venditti, N. Mantel (Biometry Branch), Dr. R. A. Darrow (now at National Institute for Medical Research, Mill Hill, London, England.

Cooperating Units:

(a) General Medicine Branch, NCI: Dr. E. Frei and staff. Serial No. NCI 226.

(b) Laboratory of Biology, NCI: Dr. J. Moloney. Serial No. NCI 420.

(c) Laboratory of Neuroanatomical Sciences, NINDB: Dr. J. Cammermeyer. Serial No. NINDB EP-1.

(d) Graduate School, Biochemistry Department, Brandeis University, Waltham, Massachusetts: Dr. Nathan O. Kaplan and staff; Chemistry Department, Dr. Orrie Friedman.

(e) Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri: Dr. R. Burton and Dr. R. Salvador.

(f) Department of Pharmacology, Tufts University Medical School, Boston, Massachusetts: Dr. M. Friedkin, Dr. L. Shuster.

(g) Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma: Dr. P. Condit.

(h) University of Miami Medical School, Miami, Florida: Dr. J. Colsky.

(i) Cancer Chemotherapy National Service Center: Microbiological Associates, Inc., Bethesda, Maryland (Contract No. SA-43-ph-2371). Serial No. NCI 216.

Man Years (calendar year 1959):		Patient Days
Total:	13	None
Professional:	6	
Other:	7	

Project Description:

Objectives

1. Study of the mechanism of action of antitumor agents, including metabolic antagonists, alkylating agents, antibiotics, etc.

2. Synthesis of new agents.

3. Study of host-tumor interrelationships with respect to drug action.

4. Establishment of new therapeutic principles and procedures in the management of tumor growth in animals.

Methods Employed

Macrobiological assay procedures involving methods of inhibition analysis for animals with and without tumors. In vivo and in vitro biochemical procedures. Radioactive tracer techniques. Organic chemistry procedures.

Major Findings

1. Survivors of Systemic Leukemia (with Humphreys, Venditti, Chirigos, and Chapman): It had been reported last year that the dihalogenated derivative of amethopterin, 3',5'-dichloroamethopterin (DCM), was capable of eliciting extensive increases in survival time of CDBA mice with advanced leukemia, and that some of the mice actually survived for an indefinite period of time.

A study was conducted to determine the relationship between duration of therapy and antileukemic effect with DCM. Survivors of systemic disease were obtained only when adequate doses were administered over an extended period of time. The length of time necessary for daily treatment to be continued before any significant number of survivors could be obtained proved to be 30 to 60 days.

When daily treatment with DCM was discontinued prior to 30 days, very few of the animals survived. As had been observed earlier with amethopterin, most of the animals succumbed within several days following discontinuance of therapy. After about 2 weeks of treatment the animals usually showed no gross evidence of the disease, and inoculation of blood or transplantation of tissues into other mice frequently failed to transmit the disease. Microscopic examination of the tissues of such animals might also show no evidence of the disease. That such apparently cured mice still harbor the disease was demonstrated by the regrowth of the leukemia when treatment was discontinued too soon.

2. Studies on Immunity (with Humphreys, Chirigos, Chapman, and Venditti): It had also been reported that mice surviving systemic leukemia were refractory to reinoculation of a massive dose of leukemic cells. These studies have been extended. Mice surviving systemic leukemia L1210 show transplantation immunity not only to reinoculation of the sensitive line of leukemia L1210, but also show a high degree of transplantation immunity to inoculation of an amethopterin resistant subline, M46R, (which is also markedly resistant to DCM). The survivors of systemic disease were also immune to a DCM resistant subline (M66-3R) of leukemia L1210. Following inoculation of either one of these resistant tumors (or the sensitive tumor), many of the mice showed no evidence of leukemic growth, while in others the tumor grew slowly and attained a large size at the site of leukemic inoculation, prior to death. The latter observation suggests that the disease does not readily become systemic in the mice that have acquired immunity.

Similarly, "out of strain" BALB/cAn mice immunized against leukemia L1210, without the benefit of drug therapy (merely by implanting the tumor and letting it regress) were also demonstrated to be immune to subsequent inoculation of these resistant variants. Thus, in "in strain" mice the therapy is required to elicit the immune response. In "out of strain" mice the initial implant regresses spontaneously and the mice become immune.

The above results provide a separation between tumor cell resistance to therapy as opposed to tumor cell resistance to host immunity. The two variants of leukemia L1210 (M46R and M66-3R) are resistant to antifolic therapy but are inhibited by the induced immunity of the host. Thus, although the immune mechanism may contribute to therapy in the "in strain" mice, resistance to therapy does not necessarily imply abrogation of the effectiveness of the immune response. This is in agreement with the previously reported finding that failure of therapy is not necessarily associated with the development of tumor cell resistance.

It has been observed that the homograft response to the amethopterin resistant subline L1210-M46R may be overcome by treatment of the "out of strain" mice with amethopterin or DCM. On treatment with these drugs the tumor grows progressively and the animals succumb. However, if the "out of strain" mice were immunized by preinoculation of normal spleen of DBA mice or with leukemic tissue, antifolic treatment did not suppress the established immunity.

The above observations raise questions concerning the role that host immunity may play in influencing the host-tumor relationship in chemotherapy. To what extent is the antileukemic action of the antagonist attributable to immune responses influencing host tumor compatability relationships? That the successfully treated leukemic mice show evidence of immunity would suggest that at least part of the curative action of the halogenated derivatives results from therapy so influencing the host-tumor relationship that the mice develop immunity to the disease. The immune state in turn may help to eradicate the disease. If true, what are the detailed mechanisms and can advantage be taken of this in therapy?

3. Sensitization of a Resistant Tumor to Therapy (with Humphreys, Chirigos, Venditti, and Chapman): A model experiment has been conducted showing that it is possible to sensitize an antifolic resistant subline of leukemia L1210 to therapy. This was accomplished by prior inoculation and treatment of the sensitive line with DCM. The tumor employed was the M46R resistant variant of leukemia L1210, which is highly resistant to methotrexate, and markedly cross-resistant to DCM. M46R controls live for about 10 days. Treatment with DCM, even when treatment is initiated on the day of implant, extends the survival time only to approximately 20 days. The survival time of the mice with this resistant tumor may be increased to as much as 60 days if the animals are preinoculated with the sensitive leukemia and treatment with DCM is initiated after the disease has become systemic.

Presumably the treatment of the mice with the sensitive systemic leukemia elicits an immune response in the host which in turn, contributes to the therapy of the resistant leukemia. This presumption would appear to be warranted, since mice that had survived systemic leukemia L1210 following extensive therapy (day 7 to 90) were demonstrated to be wholly or partially immune to reinoculation of either the sensitive or antifolic resistant variant. The possibility is not excluded, however, that the sensitive leukemic cells may have sensitized the resistant cells directly or following therapy. The study indicates that the chemotherapy of combinations of sensitive and resistant variants may be employed to study immunological and other factors pertaining to transplantation, and to investigate means for the improvement of therapy of resistant variants. Resistant sublines may serve as convenient markers for studying these interrelationships.

4. Route of Administration of Antifolics

a. Survival time (with Venditti): The extensive survival time observed on treatment of mice with advanced leukemia was obtained with subcutaneous administration of DCM. A study has now been conducted showing that on oral administration of DCM, there is a marked loss in antileukemic effectiveness. Amethopterin has also shown reduced effectiveness on oral administration.

b. Effect of route of antifolic administration on formate incorporation (with Schrecker, Mead, and Lynch): Since the inhibition of formate incorporation into the acid-soluble adenine of leukemic spleens can serve as a measure of effective levels of antifolic drugs, in vivo, it was employed to compare the effectiveness of different routes of administration. In leukemic mice, the subcutaneous and intraperitoneal routes produced similar levels of inhibition with DCM. Maximum inhibition was observed when formate was injected 20 minutes after the administration of the drug. Even after 7 minutes, near-maximal inhibition was obtained. However, with oral administration of DCM, maximum inhibition was not reached before an hour. Moreover, much larger doses were required to produce inhibition levels comparable to the ones found on parenteral administration. It would appear, therefore, that DCM is only slowly absorbed from the intestinal tract, and that much larger doses are needed to produce an antitumor effect than by the parenteral route. Since one of the main toxic effects of DCM is that produced in the intestinal tract, our studies would suggest that the oral route of administration is inferior. This is of importance in its implications for clinical therapy.

5. New Antileukemic Agents (with Venditti and Humphreys). Cytoxan, a cyclic phosphamide ester of nitrogen mustard, was investigated in some detail against leukemia L1210 and several of its resistant sublines. It was found to be more effective than a series of alkylating agents including uracil mustard, nitrogen mustard, triethylene melamine, degranol, chlorambucil, hydroquinone mustard, sarcolysin, or nitromin, in increasing the survival time of mice with advanced leukemia. It was at least as effective as amethopterin. In agreement with data of Dr. M. Lane, its antileukemic effectiveness against advanced leukemia was demonstrable over a wide range of schedules of therapy. Intermittent administration of the drug appeared to provide more effective therapy.

The antitumor effectiveness of Cytoxan was not diminished in the treatment of sublines of leukemia L1210 which were resistant to the antifolics or the antipurines. The antileukemic action of Cytoxan could not be blocked by nicotinamide, as had been reported elsewhere.

Studies with Cytoxan against early transplantable leukemia L1210 suggest that the action of this alkylating agent results in the actual destruction of antileukemic cells. A single dose of Cytoxan was so effective against early leukemia that many of the mice survived.

By comparison with the treatment of normal mice, it was demonstrated that the therapeutic effectiveness of Cytoxan was limited by the toxicity of the drug for the host. This toxicity was less limiting than had been previously observed with 5-fluorouracil.

Cytoxan was also demonstrated to be effective against adenocarcinoma 755 in mice, retarding the growth of the local tumor, and increasing the survival time of the mice.

Cytoxan derivatives - A collaborative research program is in progress with Dr. Orrie Friedman at Brandeis University, who is synthesizing congeners of Cytoxan. Structure-activity relationships are under investigation with these compounds in this laboratory.

6. Pyrazolo Pyrimidines (with Venditti and Frei): A detailed study was conducted on the effectiveness of the pyrazolo(3,4-d) pyrimidines against transplantable mouse tumors.

A series of pyrazolo(3,4-d) pyrimidines, substituted in the 4 position, was compared with 6-mercaptopurine and amethopterin for ability to increase the survival time of mice with advanced leukemia L1210. There was a wide range of antileukemic activity among the various pyrazolo pyrimidines employed, but none of them was as effective as 6-mercaptopurine or amethopterin. 4-Aminopyrazolo (3,4-d) pyrimidine (4APP); 4-n-propylaminopyrazolo(3,4-d) pyrimidine; 4-benzylaminopyrazolo(3,4-d) pyrimidine; and 4-amino-1-methyl pyrazolo(3,4-d) pyrimidine were the most effective of the pyrazolo pyrimidines tested. They were approximately 30 per cent as effective as amethopterin in increasing survival time of mice with advanced leukemia.

4APP failed to produce appreciable increases in the survival time of mice with an 8-azaguanine resistant subline of L1210, also resistant to 6-mercaptopurine. On the other hand, 4APP did produce marked inhibition of growth of the local tumor in mice bearing the antipurine resistant leukemia, indicating that cross resistance was not complete.

4APP and the 4-benzyl derivative inhibited the growth of relatively early adenocarcinoma 755, but failed to increase the life span of the mice. In contrast, 6-mercaptopurine produced a marked increase in the survival time of the mice bearing adenocarcinoma 755 and also resulted in a high incidence of tumor free survivors.

4APP was ineffective against sarcoma 37. It failed to inhibit local tumor growth and failed to increase the survival time of the mice.

7. Evaluation of Antileukemic Agents Employing Advanced Leukemia L1210 in Mice (with Venditti, Kline, and Mantel): The assay procedure employing advanced leukemia in mice, developed in this laboratory, had originally been employed to evaluate the antileukemic efficacy of 38 compounds, including 23 that had been employed in the Gellhorn-Hirschberg report, on investigation of diverse systems for Cancer Chemotherapy Screening. This assay system is now in full-scale use in the Drug Development and Evaluation Program (Contract No. SA-43-ph-2371). A total of 125 compounds has been evaluated in this system with respect to their relative capacities to extend survival time of mice with leukemia L1210. Included in this list are alkylating agents, antibiotics, purine and pyrimidine antagonists, nicotinamide antagonists, colchicine derivatives, etc. Two solid tumor assay systems have also been developed (Adenocarcinoma 755 and Sarcoma 37), and are currently being employed in the Drug Development and Evaluation Program (details are listed in report on Contract SA-43-ph-2371).

8. Virus-induced Leukemia (with Moloney and Humphreys): A collaborative program has been instituted with Dr. J. Moloney to study the effect of chemotherapeutic agents against the Moloney virus leukemia. Moloney obtained this virus from sarcoma 37. It induces lymphocytic leukemia in mice, and is not strain specific in this regard. The induced leukemia is transplantable as the whole cell, but is strain specific. Sufficient studies have been conducted to date in this program to demonstrate that the virus induced leukemia provides a useful tool in chemotherapeutic studies. Once the disease has developed it appears to be highly refractory to

therapy. Little or no increase in survival time was obtained with amethopterin, Cytoxan, or 6-mercaptopurine. On the other hand, definite reduction was observed in the size of the spleen, liver, thymus, and nodes following therapy with these drugs. This work is to be extended to include study of the action of drugs on (a) the virus itself, (b) the induced leukemia, and (c) the transplanted leukemia. Treated animals are being examined for microscopic pathology by Dr. Thelma Dunn.

9. Spontaneous Tumors (with Humphreys, Chirigos, and Chapman): Chemotherapeutic studies have been undertaken with spontaneous mammary tumors in C3H mice. Although the initial study has not been completed, it is already evident that the spontaneous tumor is highly refractory to amethopterin, 6-mercaptopurine, and Cytoxan.

10. Combination of Chemotherapy and Surgery (with Colsky, Chirigos, and Humphreys): Some evidence has been obtained that the combination of surgery plus treatment with 6-mercaptopurine is more effective than treatment alone, in inhibiting tumor growth and possibly in increasing the survival time of mice with adenocarcinoma 755.

11. Biochemical Studies with Antifolics.

a. Inhibition of purine synthesis (with Schreiner, Mead, Darrow and Lynch): Studies were continued on the inhibition of purine synthesis de novo in normal and leukemic mice by amethopterin and 3',5'-dichloroamethopterin (DCM), as measured by the inhibition of formate-C^{14} incorporation into acid-soluble adenine of spleen and other organs. A small parenteral dose of either drug produced extensive inhibition in spleen or leukemic tissues when it was administered less than one hour before the injection of labeled formate. For instance, a single dose of 1.5 mg/kg of DCM produced over 90 per cent inhibition in leukemic spleens when it was given 20 minutes before administration of formate. With this 20-minute interval, the dose ratio of DCM to amethopterin for equal inhibition, as determined in dose-response studies, was about 2. However, the inhibitory effect of the drugs was transitory, and the effect of DCM was much less persistent than that of amethopterin. By extending the interval between the administration of drug and of formate, recovery curves were obtained. They showed

that, with an interval of 6 hours or more, the dose ratio of DCM to amethopterin for equal inhibition, became about 35 to 1. It should be noted that, to obtain inhibition lasting for 24 hours, about 75 mg/kg of DCM was required. This is the dose that produces maximum survival times in leukemic mice on daily treatment. The finding indicates that, in therapy, it is necessary to maintain an effective dose-level between consecutive injections.

By measuring dose-response relationships with amethopterin and DCM in both normal and leukemic spleens, it was found that DCM was relatively more effective than amethopterin in the latter. This may account for the more favorable effect of DCM in prolonging life in leukemic mice.

b. Mechanism of reversal by citrovorum factor (with Schrecker, Mead, and Lynch): Treatment with very large amounts of citrovorum factor (CF) caused partial reversal of the inhibitory effect of amethopterin and DCM upon formate incorporation into the acid-soluble adenine of leukemic and normal spleens in vivo. Increased doses of CF were needed with increased doses of the drugs. Reversal was more pronounced when CF was administered before, rather than after, injection of DCM. The current theory of the action of folic acid antagonists does not appear to explain these results. Since amethopterin blocks the formation of tetrahydrofolic acid from folic acid, and since CF can yield tetrahydrofolic acid by a different pathway, supposedly unaffected by amethopterin, formate incorporation should not be inhibited at all by amethopterin if sufficient CF is supplied. Our results show that this is not the case. Therefore, amethopterin (and DCM) must inhibit the transformylation reactions in purine nucleotide biosynthesis or inhibit the transport of CF into cells. The latter has been demonstrated by Wood and Hitchings in bacteria. Our study suggests that this may also be true for leukemic cells and for normal spleen cells.

c. Correlation of biochemical indices with therapy (with Schrecker, Mead, and Humphreys): Normal liver was found to be rather insensitive to antifolic drugs. Thus, a dose of 75 mg/kg of DCM failed to produce inhibition of formate incorporation in that organ. Since formate incorporation in leukemic cells is much higher than in liver, and since it

is greatly inhibited by DCM, it can serve as a measure of systemic infiltration during the leukemic process as affected by treatment. Formate incorporation was measured in leukemic mice that were treated daily with 75 mg/kg of DCM from day 7 to 17 after inoculation. In untreated control mice, formate incorporation rose sharply as the livers became infiltrated. Treatment reduced incorporation to the levels observed in normal livers. With cessation of treatment, incorporation rose sharply again after a delay of about 3 days, indicating reinfiltration of the livers with leukemic cells. Analogous results were observed in spleen.

d. Resistance studies (with Schrecker and Mead): Studies have been instituted on amethopterin-induced inhibition of formate incorporation in an amethopterin-resistant subline of L1210 leukemia (MW6R). Large doses produced extensive inhibition of formate incorporation in leukemic spleen and local tumor with the sensitive and resistant subline. With small doses, inhibition was less pronounced in the resistant subline. The differential effect was more pronounced in the local tumor than in the infiltrated spleen. The data suggest that the dose-response curve for inhibition is shifted toward the larger doses in the resistant subline, in agreement with Nichol's description of resistant cell populations. The difference between the sensitive and the resistant leukemia would appear to be of a quantitative rather than qualitative nature.

e. Dihydrofolic reductase (with Dr. Friedkin, Humphreys, and Schrecker): Studies are in progress on the inhibition of dihydrofolic reductase by antifolics in sensitive and resistant tumors. Evidence has been obtained that dihydrofolic reductase activity is higher in the resistant subline studied than in the sensitive leukemia.

12. Amino Acid Metabolism

a. Tyrosine and Tryptophan (with Chirigos): Preliminary studies have shown that tryptophan and tyrosine are present in significant amounts in tumor as well as in normal tissues. A survey is being made of endogenous levels of tyrosine and tryptophan in tumor tissue as compared to normal tissue.

b. Transaminase (with Chirigos): Transaminase activity is being surveyed in a series of transplantable and spontaneous tumors. Several amino acids are being studied to determine whether individual transaminases exist for each essential amino acid.

c. Transport (with Chirigos): Transport of aromatic amino acids in solid tumors is being investigated in relation to inhibition by alkylating agents such as the phenylalanine mustards.

A system of growing leukemia in mouse brain has been developed in order to ascertain whether drugs effective against this tumor depend on a transport mechanism to exert their antileukemic effect.

13. Pyridine nucleotide Synthesis (with Narrod, Langan, and Kaplan): The injection of azaserine was demonstrated to inhibit markedly the synthesis of diphosphopyridine nucleotide (DPN) resulting from nicotinamide injection.

The amount of DPN in mouse liver is increased markedly on injection of a number of pyridine derivatives, of which nicotinamide has been demonstrated to be most effective, raising the level by 8-10 fold. 6-Mercaptopurine, thioguanine, and nicotinic acid inhibit the synthesis of DPN. Azaserine, unlike the other inhibitors of this system, actually caused a marked reduction in the normal levels of the pyridine nucleotide. There was an approximately 9-fold decrease of pyridine nucleotide level, as compared with the level in normal animals. The maximum decrease occurred in 1-2 hours and recovery occurred in approximately 24 hours. 6-Diazo-5-oxo-L-norleucine (DON) behaved similarly to azaserine in lowering mouse liver DPN. Since azaserine and DON are known to inhibit nucleotide synthesis of precursors of DPN, this may account for the inhibition of DPN synthesis. Additional investigations are in progress and it is hoped that these studies will be of value in elucidating the pathway of DPN synthesis and its regulation in the intact animal.

14. Formation of Nicotinic Acid Analogue of Diphosphopyridine Nucleotide Following Nicotinamide Administration (with Langan, Kaplan, and Shuster): A pyridine nucleotide

isolated from the livers of mice injected with nicotinamide has been identified as the nicotinic acid analogue of DPN. Evidence has been obtained that it is an intermediate in the synthesis of DPN from both nicotinamide and nicotinic acid, as well as from other pyridine compounds. Inhibition by azaserine of DPN synthesis from nicotinamide allowed some accumulation of nicotinic acid analogue.

15. Interaction of Nicotinamide with Reserpine and Chlorpromazine (with Burton, Salvador, and Humphreys): Nicotinamide will act in conjunction with reserpine and chlorpromazine to reduce the spontaneous activity of mice and to increase the duration of anesthesia.

16. 3-acetyl pyridine toxicity (with Cammermeyer and Humphreys): Studies are in progress to investigate the pathology of the ataxia associated with administration of 3-acetyl pyridine.

Significance to the Program of the Cancer Institute:

These studies are designed to provide basic information pertaining to the host-tumor relationship in chemotherapy, from both a pharmacological and biochemical point of view. From such studies, principles for treatment in animals are being established which may be of use in application to clinical chemotherapy.

Proposed Course of Project:

During the next year the various phases of this work will be continued and broadened. Particular emphasis will be placed on phasing into the newly undertaken areas of research, indicated above.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) Shuster, L., Khorana, H. G., and Heppel, L. A.: The Mode of Action of Ryegrass Ribonuclease. Biochim. Biophsica Acta 33, 452-461, 1959.

(2) Goldin, A., Venditti, J. M., Humphreys, S. R., Shuster, L., Darrow, R. A., and Mantel, N.: Advanced Leukemia as a Tool for Chemotherapeutic Studies. In press, ACTA UNIO INTERNATIONALIS CONTRA CANCRUM, (Proceedings of 7th International Cancer Congress, London, England, July 6-12, 1958). (Reported in last years' report)

(3) Liebling, M., Humphreys, S. R., and Goldin, A.: Studies of 5-Fluorouracil in the Treatment of L1210 Leukemia in Mice. Cancer Research 19, 116-121, 1959.

(4) Shuster, L., and Goldin, A.: Some Biochemical Effects of 2-Ethylamino-1,3,4-thiadiasole. Biochemical Pharmacology 2, 17-24, 1959.

(5) Shuster, L., and Goldin, A.: The Effect of Nicotinamide on In Vivo Incorporation of Formate-C^{14}. J. Biol. Chem. 234, 129-133, 1959.

(6) Goldin, A., Humphreys, S. R., Venditti, J. M., and Mantel, N.: Prolongation of the Lifespan of Mice with Advanced Leukemia (L1210) by Treatment with Halogenated Derivatives of Amethopterin. J. Nat. Cancer Inst. 22, 811-823, 1959.

(7) Humphreys, S. R., and Goldin, A.: Investigation of Tumor Variants Recovered from Mice with Systemic Leukemia (L1210) Following Extensive Therapy with 3',5'-Dichloroamethopterin and 3', Bromo-5'-Chloroamethopterin. J. Nat. Cancer Inst. 23, 633-653, 1959.

(8) Laszlo, J., Humphreys, S. R., and Goldin, A.: Effects of Glucose Analogs (2-Deoxy-D-glucose, 2-Deoxy-D-galactose) on Experimental Tumors. J. Nat. Cancer Inst., in press.

(9) Goldin, A., and Humphreys, S. R.: Studies of Immunity in Mice Surviving Systemic Leukemia L1210. J. Nat. Cancer Inst., in press.

(10) Venditti, J. M., Humphreys, S. R., and Goldin, A.: The Effectiveness of Cytoxan Against Mouse Leukemia L1210 and Resistant Sublines. Cancer Chemotherapy Reports 4, 6-8, May, 1959.

(11) Venditti, J. M., Humphreys, S. R., and Goldin, A.: Investigation of the Activity of Cytoxan Against Leukemia L1210 in Mice. Cancer Research 19, 986-995, 1959.

(12) Harrod, S. A., Langen, T. A., Jr., Kaplan, N. O., and Goldin, A.: Effect of Azaserine (o-diazoacetyl-L-serine) on the Pyridine Nucleotide Levels of Mouse Liver. Nature 183, 1674-1675, 1959.

(13) Charache, S., Condit, P. T., and Humphreys, S. R.: Studies of the Folic Acid Vitamins. IV. The Persistence of Amethopterin in Mammalian Tissues. In press, CANCER.

(14) Charache, S., Condit, P. T., Levy, A. M., Humphreys, S. R., and Goldin, A.: Studies on the Folic Acid Vitamins. V. Effect of Folic Acid on the Citrovorum Factor Activity of Mouse Liver. In press, CANCER.

(15) Langen, T. A., Jr., Kaplan, N. O., and Shunter, L.: Formation of the Nicotinic Acid Analogue of Diphosphopyridine Nucleotide Following Nicotinamide Administration. J. Biol. Chem. 234, 2161-2168, 1959.

(16) Goldin, A., Venditti, J. M., Kline, I., and Hankel, [illegible]: Evaluation of Antileukemic Agents Employing Advanced Leukemia L1210 in Mice. Cancer Research 19, Cancer Chemotherapy Screening Data IV, 429-466, November, 1959.

(17) Venditti, J. M., Frei, E., and Goldin, A.: Effectiveness of Pyrazolo(3,4-d) Pyrimidines Against Transplantable Mouse Tumors. In press, CANCER.

Honors and Awards relating to this project:

A. Goldin: (a) Received reappointment as Visiting Professor of Biochemistry at Brandeis University, Waltham, Massachusetts; (b) Panel Member, Board of U. S. Civil Service Examiners at NIH; (c) Foreign Corresponding Member Societa Italiana Di Cancerologia; (d) Affiliate membership, Royal Society of Medicine; (e) Appointed to Cancer Chemotherapy National Service Center Drug Evaluation Panel, and to its Committee on Experimental Therapeutics; (f) Appointed to Cancer Chemotherapy Study Section, Division of Research Grants, NIH; (g) By invitation, served on Examining Committee for consideration of Sachindra Nath Pradham as candidate for Ph.D. degree, January 28, George Washington University School of Medicine; (h) Invited to present work of the laboratory at Inter-departmental Research Seminar, University of Maryland School of Medicine, March 4; (i) Invited to present work of the laboratory at University of Pittsburgh Medical School, May 4 and 5; (j) Nominated to attend Public Health Service Orientation Conference for Commissioned and Civil Service Personnel, May 11-12; (k) By invitation, presented data to, and participated in, New Agents Committee Clinical Study Panel, June 16; (l) Together with General Medicine Branch, NCI, presented work of the laboratory on closed circuit television broadcast, July 31; (m) Invited to participate in symposium on "Comparison of Present Status of Chemotherapy of Transplanted Rodent Tumors and the Chemotherapy of Human Tumors, Mt. Desert Island Biological Laboratory, Bar Harbor, Maine, August 10-11; (n) By invitation, participated in CCNSC Conference on Experimental Clinical Cancer Chemotherapy, November 11-12.

J. Venditti: (a) By invitation, participated in Gordon Research Conferences on Cancer, New London, New Hampshire, August 31-September 4.

A. Schrecker: (a) Invited to spend a month as guest worker in the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts.

Serial No. NCI-252
1. Lab. Chemical Pharmacology
2. Cellular Pharmacology Section
3. Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pharmacologic Action of Tumor-Damaging and other Chemical Agents on Animal and Human Malignant and Normal Cells.

Principal Investigator: Dr. M. Belkin

Other Investigators: Dr. J. W. Preiss, Dr. D. L. Vivian, A. Perrault.

Cooperating Units: Dr. D. Lavie, Weizmann Institute, Israel. Dr. A. J. Dalton, Cellular Biology Section, NCI-406.

Man Years (Calendar Year 1959):		Patient Days:
Total	6	None
Professional	1	
Other	5	

Project Description:

Objectives:

(a) To study tumor necrotizing compounds, from plant and other sources, to determine how they bring about cell damage and cell death. (b) The morphologic and biochemical responses of normal and malignant cells to various drugs and chemical agents with particular regard to certain basic functions of the cell, i.e., permeability, water metabolism, enzyme behavior, etc.

Methods Employed:

Classical techniques of pharmacology, cyto- and histochemistry, and tissue culture are used. New techniques are developed as necessary.

Major Findings:

For each of the several facets of this project, the principal findings and general progress during the past year are as follows:

A. Tumor Damaging Capacity of Plant Materials.

The systematic testing of crude plant materials for tumor damaging capacity has now been discontinued.

The results of the final tests have been embodied in two manuscripts, as follows:

1) 27% of 105 plant materials from 82 species of Australian medicinal and poisonous plants induced damage in transplanted sarcoma 37. Four plants, arbitrarily selected, were also screened against seven additional transplanted mouse tumors. Each of the four plants inflicted damage upon a number of tumors, but not always the same tumors. A certain degree of selectivity appears indicated.

2) Of a series of 65 plant materials from 58 plant species commonly used as foods, 34% produced damage in sarcoma 37.

When the polysaccharides, dialyzed from a number of these food plants, were tested against solid sarcoma 37, pronounced tumor hemorrhage and necrosis was produced. Also, these polysaccharides could induce typical enlargement and vacuolization of sarcoma 37 and other ascites tumor cells (see below).

These marked effects produced by these polysaccharides were obtained only after parenteral administration (I.P. or I.V.). Non-absorption or inactivation in the gut appears to be quite complete; since no effect was obtained when polysaccharide was given orally, even at

doses ten times the M.T.D.

B. Studies in Cellular Pharmacology.

I. Factors Regulating the Control of Intracellular Water Metabolism.

In the previous Annual Report, there was described the production of 3 types of blebs in ascites tumor cells when they were exposed to certain chemical agents. The form of these blebs were (1) acentric (2) symmetrical and (3) scallop (numerous small blebs which border the cell).

This study has been extended to include six additional mouse and one rat ascites tumors. The cells of all these tumors responded with the formation of one or the other of the 3 types of blebs, depending on the agent used.

The chemicals capable of producing cell blebs all have the property of reacting with sulhydryl groups. Such reaction is of 3 possible kinds: by alkylation, by oxidation or by mercaptide formation.

It is of interest that the majority of chemicals producing blebs are those which form mercaptide-linkages, with a few exceptions: iodoacetamide, an alkylating agent produced scallop blebs; hydrogen peroxide, an oxidizing agent, produced acentric blebs; and $KMnO_4$, $CuCl_2$ and porphyrindine, also oxidizing agents, produced symmetrical blebs.

The capacity to produce blebs when acted on by certain chemical agents appears to be a general property of malignant ascites cells. To date, cells of 5 normal tissues, exposed to appropriate agents, have failed to react in this manner.

Two implications may be drawn at the present time:

1. blebs are formed as a result of inhibition of suldryl-containing enzymes (one of which is succinic-dehydrogenase) which is associated with a disturbance in

the water regulating capacity of the cell.

2. there appear to be structural and functional differences between the cell membrane of malignant cells and (several) normal cells, which are responsible for their different behavior when exposed to blebbing agents.

II. Studies on Vital Staining with Neutral Red.

This program, concerned with the synthesis and testing of new congeners of neutral red, has been terminated.

A manuscript, describing the preparation and properties of some 2,8-disubstituted phenazines used as intermediates plus 2 new vital stains related to neutral red, has been accepted for publication in the Journal of Organic Chemistry (see below).

III. Evaluation of Vital Stains as Indicators of Cell Injury and Death.

Investigation as to the validity of vital stains as indicators of viability has continued.

The behavior of several vital dyes, and other dyes not generally considered as such, was observed with motile protozoan cells as test objects (paramecium, colpidium). Experiments were designed so that optimal concentrations of stain and toxic agent allowed death with sufficient slowness so that the behavior of the dye could readily be followed.

Observations to date permit a number of tentative conclusions:

1. Vital stains may vary considerably with regard to speed in indicating cellular damage, i.e., eosin penetrates faster than nigrosin, etc.

2. The behavior of vital stains (like neutral red and crystal violet) which concentrate in the cell interior in localized areas appears to depend more

upon the condition of the localized stained area than upon the total physiological state of the cell. This type of stain is comparatively sluggish in indicating cell damage.

3. Loss of the property of selective permeability to a dye appears to be a more sensitive criterion and takes place rapidly to indicate cell damage (eosin, trypan blue, brilliant vital red and fast green stain faster than neutral red).

4. Some toxic agents allow penetration of certain stains (eosin, fast green) even though the cells are still motile and appear relatively undamaged.

IV. Studies with Human Malignant Cells in Tissue Culture.

As part of the program concerned with the nature of cell damage by chemical agents, the effect of Suramin Sodium (Naphuride Sodium) was studied on cultures of HeLa, D189, D164 and D227.

After exposure of these cultures to Suramin, 1 mg/ml. for 24-48 hours, the following changes were observed:

1) Disruption of the early reticular pattern of growth.

2) Fragmentation or "beading" of the cell membrane and cytoplasm.

3) Retraction of cytoplasmic processes.

4) A population of predominantly rounded cells.

Although all the cell lines exhibited the above qualitative changes, the time required to evoke these changes among them varied considerably, with D-227 showing the greatest sensitivity. The various rates of response to Suramin and other chemical agents like hydrocortisone are being exploited to determine whether by this means it will

be possible to distinguish these 4 cell lines one from the other, a most difficult thing to do on purely morphologic appearance, and a desirable objective.

V. Effect of Higher Plant Polysaccharides on Ascites Tumor Cells.

As described previously, many plant polysaccharides will, when given I.P. to mice with ascites tumors, cause swelling and vacuolization of the cells. The initial findings have been published (see below).

Further experiments have indicated the indispensable role of the host in eliciting this phenomenon.

1. Sarcoma 37 ascites cells were exposed in vitro to golden rod polysaccharide for one hour, then washed 3 times in balanced salt solution and injected I.P. into normal mice. Marked cell vacuolization was evident in less than 20 hours.

2. Normal mice were injected I.P. with golden rod polysaccharide. After intervals of 30 minutes to 72 hours after such administration, the mice were injected I.P. with sarcoma 37 ascites cells, and beginning 4 hours later, cell samples were examined. Vacuolization was evident when the cells were inoculated 1 hour after polysaccharide injection; increased up to intervals of 36 hours after polysaccharide injection (depending on the dose) and then fell sharply after this period.

3. Mice carrying sarcoma 37 ascites tumor were injected I.P. with golden rod polysaccharide. At varying time intervals, i.e., from 1 to 7 hours after polysaccharide injection, the cells were removed, washed 3 times with balanced salt solution, and then transferred to new, normal hosts. These cells were then examined for the next 96 hours.

This experiment disclosed that cells exposed <u>in vivo</u> to polysaccharide for as brief a period as 90 minutes, and then transferred to a new, non-treated host

would go on to produce a good vacuolization effect in 24-48 hours.

A number of implications follow from these experiments:

1. The polysaccharide is adsorbed on the cell surface, when the cell is in contact with it.

2. The cells need not come into prior contact with the polysaccharide in order to produce vacuolization.

3. When normal hosts are injected with polysaccharide, they are "stimulated" in some fashion and can induce vacuolization in cells transplanted I.P. as long as 36 hours later, when presumably little or no polysaccharide remains as such.

4. The participation of the host is indispensable to the induction of cell vacuolization and swelling.

Experiments are in progress to determine whether the host produces antibodies in response to polysaccharide administration in a manner similar to that established for the polysaccharides of Gram-negative bacteria, and whether such a substance reacts with the polysaccharide at the cell surface (see 1, above). Also to determine more closely which organ system of the host participates in this phenomenon.

It may be conjectured that these vacuoles arise from vacuoles already present in the cell, or de novo from cellular structures which might give rise to them, such as the endoplasmic reticulum.

Preliminary studies with the electron microscope (with Dr. Dalton) indicate that these vacuoles arise from pre-existing vacuoles, some of which surround the virus particles known to be present in this tumor.

VI. Electron Inactivation of Plant Polysaccharide.

Polysaccharide, obtained from golden rod flower, was exposed in vacuo to 7 K.V. electrons, emitted by a hot tungsten filament and accelerated by a grid arrangement. Exposures ranged from 9×10^{13} electrons/cm^2 to $50 + 10^{13}$ electrons/cm^2. Control samples of polysaccharide were similarly handled but not radiated. (These procedures were carried out by Dr. Preiss). Both treated and untreated polysaccharide was injected I.P. into mice with 3-day sarcoma 37 ascites; and cells were examined every day for 4 days.

The polysaccharide controls always exhibited typical cell vacuolization. The treated samples, however, displayed progressive inactivation with increasing dose, from 46% inhibition of the vacuolization phenomenon at the lowest electron exposure, to 98% inhibition at the highest.

It has been calculated, that the degree of inhibition of golden rod polysaccharide by the electron source employed approximates the expression from the target theory of single hit inactivation, the formula for which is

$$A = Ao\ e^{-DV}$$

where A = residual biological activity after irradiation

Ao = biological activity of controls

D = dose expressed as ionizations/cc

V = is the apparent target volume.

It was found that it takes about 16 times as many electrons to attenuate the activity of the golden rod polymer by a given percent as it does to attenuate the sucrose hydrolyzing activity of invertase to the same degree. Since the material densities are about the same, the molecular weight of the active moiety of golden rod polysaccharide must be of the order of 7500.

Comparison molecular weights obtained by this electron beam technique for the enzymes invertase and ribonuclease are 110,000 and 15,000; whereas values for the same substances obtained by conventional methods are 120,000 and 13,500, respectively.

Significance to the Program of the Cancer Institute:

These studies are designed to obtain basic information concerning (a) possible differences between normal and malignant cells, (b) the mechanisms by which necrotizing compounds produce cellular damage and (c) to correlate at the cellular level the pharmacologic action of drugs with the structural and functional responses of cells in relation to treatment. Such information is sorely needed to afford a better understanding of drug action, and to aid in the development of newer and better drugs for the use of investigators working in clinical chemotherapy.

Proposed Course of Project:

Most phases of this project will be continued, with emphasis on (1) investigation of the factors regulating water metabolism in the cell, (2) the nature of cell damage due to chemical agents and (3) the development of improved tools with which to recognize such damage.

Part B included ⟹ Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) Leighton, J., Kalla, R. L., Kline, I., and Belkin, M.: Pathogenesis of Tumor Invasion I. Interaction between Normal Tissues and "Transformed" Cells in Sponge-Matrix Tissue Culture. Cancer Research 19: 23-27 (1959).

(2) Lavie, D., Willner, D., Belkin, M., and Hardy, W. G.: New Compounds from Plants with Anti-Tumor Activity. Acta XV bis: 177-181 (1959).

(3) Belkin, M., Hardy, W. G., Perrault, A., and Sato, H.: Swelling and Vacuolization Induced in Ascites Tumor Cells by Polysaccharides from Higher Plants. Cancer Research 19: 1050-1062 (1959).

(4) Vivian, D. L., Hogart, R. N., and Belkin, M.: Phenazine Syntheses. X. 2,8-Disubstituted Phenazines Made as Intermediates for New Vital Stains, together with Two New Vital Stains Related to Neutral Red. J. of Organic Chemistry, In Press.

Honors and Awards relating to this project: None

Serial No. NCI-262
1. Lab. Chemical Pharmacology
2. Macromolecular Chemistry Sec.
3. Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Biophysical study of macromolecular interaction in the inhibition of enzymes and other biologically-active macromolecules.

Principal Investigator: P. T. Mora

Other Investigators: P. Maury, E. Morler, J. W. Preiss, J. W. Wood, B. G. Young, and M. J. Shear.

Cooperating Units: NCI, Laboratory of Chemical Pharmacology, Polysaccharide Section, Serial No. 261.

Dr. W. R. Carroll and Dr. H. A. Saroff, Section on Physical Biochemistry, Laboratory of Physical Biology, NIAMD, Serial No. NIAMD-103.

Dr. H. Cohen, Princeton Laboratories, Inc., Princeton, New Jersey.

Professor R. Wolff, Faculty of Medicine, University of Nancy, France.

Man Years (Calendar year 1959):

Total:	12
Professional:	6-1/2
Other:	5-1/2

Patient Days:

None

Project Description:

Objectives: The objective of the program of the Macromolecular Chemistry Section was twofold:

1) The fundamental study of interaction of oppositely-charged macromolecules;

2) Biophysical application of knowledge thus gained in enzyme inhibition, blocking of toxicity of certain macromolecules, inhibiting phage multiplication, etc.

Methods Employed:

The methods were selected as needed, from the fields of polymer and synthetic organic chemistry: polymerization methods, preparation of substituted derivatives, etc.; from physical chemistry: viscometry, polyelectrolyte titration, spectrophotometry, etc., and from macromolecular chemistry: ultracentrifugation, electrophoresis, chromatography, etc.; and the data were correlated with results from various enzyme assays, phage assays, toxicity determination, tumor necrosis and fever production in animals, etc.

Major Findings:

I. Enzyme inhibition: The electrostatic forces in the interactions of numerous enzymes and their substrates were studied. The activities of enzymes were blocked with synthetic charged macromolecular inhibitors which had similar but higher charge than the substrate. Under these conditions the enzymes were directed to interact with the inhibitors and formed an inactive complex. However, the enzymic activity was completely recovered when the complex was dissociated by adding a second macromolecule which had a strong and opposite charge to the inhibitor macromolecule. The inhibitor was thus removed from the complex and the enzyme was now free to approach the substrate.

In these studies it was our plan to learn about the forces which bring the enzyme and the substrate together, by competing with and overcoming these forces with the introduction of a third, synthetic component. The enzyme inhibitors were prepared by adding charged groups such as sulfate or carboxyl groups to the synthetic polysaccharides. The synthetic polysaccharide derivatives can be prepared with great variety in size, structure, charge, etc. With their help we intended to learn something about the nature and the strength of forces which exist between enzymes and substrates in the early stages of interaction. These forces appear to be general, and in the numerous enzyme systems we have studied we always could achieve inhibition of the enzyme activity if we chose for blocking a synthetic substance which had an opposite charge to the net charge of the enzyme. This mechanism is most obvious where the substrate is also a macromolecule or when the substrate has a definite charge. A large number of enzyme substrate interactions fall under these definitions.

The system we studied most in detail was ribonuclease (RNase) and ribonucleic acid (RNA). We chose this system because the structure of the RNase is completely known. With small amounts of the synthetic anionic (negatively-charged) polysaccharide derivatives, such as polyglucose sulfate, we could completely inhibit the RNase, which is a cationic or positively-charged protein at the pH where it has the highest activity on the RNA, which is a negatively-charged macromolecule. We studied which type of polyglucose sulfate is the most effective in inhibiting the RNase and we found that the most important factor is the degree of sulfation, that is the charge density. If the degree of sulfation on the polyglucose sulfate was 3 per anhydroglucose unit, which is the maximum possible, this preparation inhibited RNase more than a thousand times more powerfully than the hitherto known best inhibitor (heparin), and several times better than less substituted polyglucose sulfates with 1 or 0.6 sulfates per anhydroglucose units.

Similar quantitative studies were done comparing inhibitory potency with charge density, using several polyglucose carboxyl derivatives. In this case too the charge density was found to be the

most important macromolecular parameter. Similar results were obtained with other enzyme systems. Therefore it appears that the most important force in the early stages of interaction between these enzymes and the substrates is probably electrostatic. It is known that only electrostatic forces can extend beyond about 5 Å between interacting macromolecules, therefore it is not surprising that these forces are operative in bringing together the enzyme and the substrate.

The molecular weight of the inhibiting substances was found to be of less importance, except that it was always necessary to have a macromolecular substance with a molecular weight of at least 2,000 to obtain inhibition. The structure was of even less importance. In all of these experiments the inhibiting synthetic polysaccharides were compact, spherical macromolecules. This allowed a study of the macromolecular interaction on a three dimensional basis which cannot be done with linear macromolecules; and this approach is opening up the possibility of determining the size of the active site of enzymes.

The RNase activity was inhibited also on the small substrate molecules cyclic uridylic and cytidylic 3-5 phosphates.

Another enzyme system which was studied in some detail was the action of lysozyme on the cell wall of Micrococcus lysodeikticus. We determined the amount of polyglucose sulfate necessary to obtain quantitative inhibition, and found it to be approximately equal to the amount of the lysozyme, which has about the same molecular weight as the inhibitor.

Other cationic enzymes which were inhibited by the synthetic anionic polysaccharide derivatives were hyaluronidase, DNase, trypsin, chymotrypsin, cytochrome-c oxidase, etc. The work on trypsin and chymotrypsin inhibition was done jointly with Dr. H. Cohen of Princeton Laboratories.

To study these phenomena, and to learn more about the fundamental nature of enzyme substrate interaction, physical chemical studies were carried out on the interaction of two oppositely-charged macromolecules. Titration of polycations with polyanions was found to follow stoichiometric equivalence. The interaction of such macromolecules was different however, from the interaction of low molecular weight ions, since a firm electrostatic complex was formed which under certain conditions such as at higher concentration led to precipitation of the macromolecular components. This phenomenon (precipitation) can be used to fractionate macromolecular components; and this principle was applied in the fractionation of a blood component, described later.

In the study of enzyme substrate interaction, the light-scattering method was also employed in the early part of the year by Dr. J. W. Preiss. Dr. Preiss, however, became more interested in applying his electron-bombardment method to biological problems, and after constructing a low energy electron gun, he studied the destruction of tumor necrotizing activity of the endotoxic polysaccharide from S. marcescens. He also applied the electron-bombardment technique to enzymes. Dr. Preiss plans to continue his experiments and interest in electron-bombardment techniques in another Institute.

II. Blocking of toxicity of cationic macromolecules.

Polymyxin, neomycin, streptomycin, or protamine, in doses which cause the death of mice within 25 to 40 minutes, can be counteracted if a neutralizing equivalent of anionic synthetic polysaccharide derivative is administered by the same intraperitoneal route 10 minutes after the injection of the toxic dose of the cationic antibiotics; the moribund mice recovered rapidly and in a few minutes appeared to be normal. This was a rapid and drastic reversion of toxic symptoms. Our findings were reported in a lead article in "Nature". If two minutes prior to the application of these cationic macromolecules polyglucose sulfate was applied at a site far from the peritoneal cavity, (under the skin of the neck), there was no toxic effect from the lethal dose of the antibiotics. Since such rapid

dissemination of the polyglucose sulfate into the peritoneal cavity is unlikely under these conditions, it is possible that the polyglucose sulfate stimulated the release of anionic polysaccharides from endogenous sources. We studied the efficiency of the different polyglucose sulfate derivatives in detoxification, and we found again that the most highly charged derivatives were by far the most active. There was a direct correlation between the ability to counteract the toxicity of cationic macromolecules and the charge density of the anionic macromolecules. Similar results were obtained with the polyglucose carboxyl derivatives.

III. Application of macromolecular interaction to the bacterial polysaccharide from S. marcescens.

The tumor-necrotizing activity of the negatively-charged polysaccharide complex preparation (Lot No. P-25) was reduced by RNase, by protamine or by other macrocations. However, when lysozyme was applied, we found unexpectedly that there was a significant increase in the tumor necrotizing property of the P-25, while at the same time it appeared that the fever producing activity was reduced. Current research is under way to study this interaction between P-25 and lysozyme and to separate, if possible, the fever producing unit of the P-25 from the tumor necrotizing portion. Ultracentrifugation experiments were carried out jointly with Dr. W. Carroll of NIAMD, using a new fractionation method, employing an analytical cell with a moving platform.

IV. Inhibition of phage T2.

Recently it was found by other investigators that the tail of the T2 bacteriophage, which attaches to the E. coli cell wall, contains a positive protein similar to lysozyme, and that the attachment happens as though electrostatic forces act between the positive tail of the phage and negative areas on the cell during the early part of the interaction. We tried to see if this electrostatic interaction could be prevented by blocking the positive tail protein with negative synthetic polysaccharide derivatives. We found that, upon short incubation at the pH of 4.6, polyglucose sulfuric acid

(or polyglucose carboxyl derivative at somewhat higher doses) completely inhibited the T2 phage, while sulfuric acid at the same pH did not reduce the viability of the phage. After these incubation steps, the mixture containing the anionic macromolecule and the phage was diluted a thousand fold and plated under normal conditions. At the lower pH of 4.2, sulfuric acid partially reduced the viability of the phage, but the effect was reversible by additional incubation at a higher pH before plating. The inhibitory action of polyglucose sulfuric acid was not reversible. These studies show that charged macromolecules have a special role in phage inhibition and demonstrate the inhibition of viruses by the introduction of a synthetic material which has a higher charge density than the receptor, and thus either denatures the virus or causes the virus to preferentially attach to the inhibitor.

During the course of our studies other polyglucose derivatives were also employed, and we inadvertently found one other type of effective inhibitor to T2 phage. At pH 9.6, molecules with two aldehyde groups were found to be effective inhibitors, and both large and small molecules caused equally good inhibition; therefore this was not a macromolecular inhibition. The aldehydes employed were glucose dialdehyde or polyglucose dialdehyde.

The T2 phage was selected for inhibition since this phage is one of the most studied viruses, and since it is easy to do quantitative work with this system. These experiments were done in preparation for extension of such inhibition studies to animal viruses.

The above experimental work, reported in sections I-IV was carried out in collaboration with B. G. Young.

V. Synthetic Polysaccharides.

In the course of preparation of negatively charged polyglucose derivatives a series of polyglucose carboxyl derivatives was prepared with different amounts of carboxyl groups per monomeric unit (P. Maury). As already mentioned, these products were used to study the charge dependence of RNase and T2 phage inhibition. Also, these preparations were found to enhance ACTH activity. This latter

work was done in collaboration with Dr. H. Cohen, Princeton Laboratories. The carboxyl derivatives are less toxic than the polyglucose sulfates which are strong anti-coagulants. The carboxyl derivatives are non-toxic i.p. at 10 mg. per mouse. Prof. R. Wolff of the University of Nancy found that the lipemin-clearing activity of the fully substituted polyglucose sulfate is several times higher than that of heparin. We are currently engaged with Prof. Wolff in a collaborative attempt to synthesize a polyglucose derivative substituted with negative groups which will have high lipemin-clearing activity and no anti-coagulant or other toxic side activity.

The different anionic polyglucose derivatives were much in demand; more than two dozen university investigators requested samples, several of them for research applications in immunology and in enzymology.

The rest of the synthetic program, which is carried out with J. W. Wood, also included preparation of positively charged synthetic polysaccharide derivatives. These cationic polyglucose derivatives are going to be used to study the inhibition of biologically active anionic macromolecules. Preliminary work showed that they are similarly effective in inhibiting enzymes with net negative charge, and they can also serve as precipitating and fractionating agents of anionic macromolecules.

The synthetic method of preparing polysaccharides from monosaccharide by chemical polycondensation procedure was extended to numerous other sugars. High molecular weight polymers of 2-deoxy-glucose, ribose, rhamnose, galactose, maltose, mannose arabinose, etc. were obtained in good yield.

The synthetic program on phenazine chemistry was terminated upon the disability retirement of Dr. D. L. Vivian.

VI. Chemical studies on the polysaccharide samples from S. marcescens.

The large scale new polysaccharide preparation of Mr. A. Perrault designated Lot No. P-45 was studied by

Dr. E. Merler. He found that the preparation is homogeneous electrophoretically but contains two components which sediment at different rates in the ultracentrifuge. The heavier component appears to be an aggregate of a lighter component with a few hundred thousand molecular weight. The P-45 is a strongly negative macromolecule, similar to P-25 . Titration of the negative groups, in collaboration with Dr. H. Saroff of NIAMD, showed that the pK_a of the negatively charged groups is about 2.7, and it is most likely to come from a carboxyl of the muramic acid type. There was only a very small amount of amino acid associated with the P-45 preparation. The great bulk of the polysaccharide is a polymer of glucose, with 0.3% phosphorus attached to it in some organically-bound form.

VII. Search for endotoxic polysaccharide complexes from mammalian sources.

Dr. H. Martin of the University of Frankfurt, reported obtaining pyrogenic preparations from human blood components such as from red cell stroma under sterile conditions, using a strong phenol extraction. However, Dr. Merler did not obtain pyrogenic material by this method. Dr. Mora, while in Europe to attend an international macromolecular symposium, visited Dr. Martin's laboratory, and together with Dr. Martin prepared a product from stroma, and currently we are attempting to reproduce the Martin method in our laboratory.

VIII. Fractionation of blood for a component which neutralizes endotoxic activity (EDC).

After the findings in the Polysaccharide Section under Dr. Landy, that human serum contains a component which inactivates the endotoxic product from bacteria, we (Mora, Young) attempted to fractionate further this factor in collaboration with Dr. Landy's group. In our fractionation scheme a selective precipitation with polyglucose sulfate was used. Upon precipitation in a narrow pH range and upon re-solution of the precipitate, most of the activity was recovered in a small fraction of the serum. Such fractionation experiments were temporarily suspended but it is planned to start this work again in collaboration with the Polysaccharide Section.

Significance to Biomedical Research and to the Program of the Institute

The enzyme blocking studies are designed to elucidate the general forces which initiate the enzyme-substrate interaction and which probably orient the enzymes' approach to the substrates so that complementary sites meet, with a very high percentage of effective collisions. Such studies are also informative with regard to the nature of the active centers of the enzymes. These fundamental studies can have practical consequences in selectively inhibiting certain enzymic activities.

The blocking of toxicity of macromolecular substances by analogous electrostatic interaction with oppositely charged macromolecules may be useful in pharmacology.

These studies of macromolecular interactions which block biological activities have importance in relation to the nature and mechanism of non-specific resistance, a subject on the program of this Laboratory and especially of the Polysaccharide Section. The fundamental work on macromolecular interaction may increase our knowledge in the above fields and stimulate future work on non-specific resistance factors, and generally on immunological factors in cancer research.

The extension of macromolecular interaction to precipitation and thus fractionation of biological entities represents a new method of separating delicate biological macromolecules under conditions where the activity is easily lost.

The phage studies give us information on the nature of the forces initiating attachment. Such forces might be similar in animal viruses. These studies are done in preparation to apply such information to the possible blocking of tumor viruses.

Proposed course of project

The fundamental studies on macromolecular interaction and the biophysical applications will be continued. Virus and enzyme inhibition will be further studied. Application of virus blocking will be attempted on some tumor virus. The macromolecular interactions related to the blocking of biological factors associated with non-specific resistance will be continued also and extended in collaboration with the Polysaccharide Section and under the direction of the Chief of this Laboratory. Also fractionation by macromolecular complexing and by other methods will be carried out for separation of blood factors participating in non-specific resistance.

Part B included: Yes

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Part B

Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) Mora, P. T. and Young, B. G.: Reversible Inhibition of Enzymes by Interaction with Synthetic Polysaccharide Macroanions. Archives Biochem. Biophys. 82, 6-20 (1959).

(2) Mora, P. T., Young, B. G. and Shear, M. J.: Reduction of Toxicity of Cationic Macromolecules by Complexing with Anionic Derivatives of Synthetic Polyglucoses. Nature, 184, 431-432 (1959).

(3) Mora, P. T. Merler, E. and Maury, P.: Synthetic Polysaccharides. IV. Preparation of Carboxyl Derivatives of Polyglucose. J. Am. Chem. Soc. 81, 5449-5451 (1959).

(4) Mora, P. T., Shear, M. J.: Reversible Inhibition of Macromolecules (Enzymes, etc.) by Electrostatic Interaction with Derivatives of Synthetic Polysaccharides. Internationale Union für Reine and Angewandte Chemie, Kommission für Macromolecülle, Symposium über Makromolekülle in Wiesbaden. Macromoleculare Naturstoffe und Modelle, Vortrage V C3, Verlag Chemie, GmbH. Weinheim, (1959).

(5) Mora, P. T., Wood, J. W. and McFarland, V. W.: Synthetic Polysaccharides. V. Polymerization of Various Aldoses. J. Am. Chem. Soc. In press. (1960).

Honors and Awards relating to this project:

P. T. Mora: a) Nominated to the NCI Specialty Fellowship Board; b) Invited by the Commission on Macromolecules, International Union of Pure and Applied Chemistry to participate in a Symposium at Wiesbaden, Germany, and to present our work on enzyme inhibition; c) Invited to give seminars at the Medical School, Yale University; Institute for Macromolecular Chemistry, University of Freiburg, Germany; C. N. R. S. Center for Macromolecular Research, University of Strasbourg, France; and Department of Biochemistry, University of Oxford, England; d) Invited to organize a symposium as Chairman on Polysaccharide Synthesis for the 138th National Meeting of the American Chemical Society, New York, 1960.

Serial No. NCI-201
1. Lab. Chemical Pharmacology
2. Polysaccharide Section
3. Bethesda 14, Maryland

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Part A.

Project Title: The role of polysaccharide complexes in resistance of the host to injurious influences.

Principal Investigator: M. Landy

Other Investigators: W. Keene, J. G. Michael, F. S. Rosen, R-J. Trapani and M. J. Shear.

Cooperating Units: Cytochemistry Section, Lab. Biochemistry, NCI, Serial No. NCI-334. Biochemistry Branch, Armed Forces Institute of Pathology, WRAMC. Rocky Mountain Laboratory, NIAID. Immunological Research Laboratory, State Bacteriological Institute, Stockholm, Sweden.

Man Years (Calendar Year 1959):		Patient Days:
Total	6 1/2	
Professional	3 1/2	None
Other	3	

Project Description:

Objectives:

To obtain information on humoral and cellular factors in the host which contribute to natural resistance to infection and to neoplasia, and the mechanisms by which these factors are influenced by polysaccharide complexes.

Methods Employed:
Biochemical, immunological, microbiological, and physiological, modified as required to meet the specific needs of particular problems.

Major Findings:
1. There has been continued interest in the extent to which the lipid and the polysaccharide moieties of bacterial endotoxins account for the characteristic host responses elicited by these complexes. A number of reports have appeared in support of the view that the endotoxic properties of these substances is attributable exclusively to their content of firmly bound lipid (lipid A). This issue has been investigated in collaboration with Dr. Edgar Ribi of the Rocky Mountain Laboratory, NIAID. Dr. Ribi has developed methods for obtaining endotoxins of low lipid content from Salmonella enteritidis by extraction with water saturated with ethyl ether. Still more of the remaining bound lipid could be removed by non-hydrolytic procedures. Endotoxin thus prepared contained only 1.2% of bound lipid in contrast to a lipid content of 19.2% in a product prepared by the traditional Boivan procedure, i.e., extraction of bacilli with trichloroacetic acid and phenol. Both products were extremely potent endotoxins. There was no significant difference in their potency as regards the following: in rabbits — capacity to prepare for the dermal Shwartzman reaction; pyrogenicity; and lethality; in mice — induction of hemorrhagic necrosis in sarcomas; and lethality. It is thus evident that a large proportion of bound lipid can be removed from bacterial endotoxin without significant reduction in its biologic activities.

2. The effect of endotoxic polysaccharides on tumor glycolysis was investigated in collaboration with Dr. M. W. Woods, Laboratory of Biochemistry, NCI. Endotoxins in concentrations of 0.003 to 0.3 parts per million effected a several-fold stimulation of aerobic acid formation from glucose by the S91 mouse melanoma. Anaerobic glycolysis was increased to a lesser extent. The degree of stimulation obtained with a series of endotoxin preparations derived from different sources by various isolation procedures paralleled their potency in eliciting typical endotoxic phenomena in vivo. The findings indicated that the properties required for elicitation of reactions in

the host by these macromolecular materials may be the same as those involved in affecting glycolysis in vitro.

3. Studies on an endotoxin-detoxifying agent (EDC) in animal tissues were continued in collaboration with Dr. Waravdekar, Head of the Biochemistry Branch at the Armed Forces Institute of Pathology. Incubation in vitro of thin slices of rabbit liver, kidney or spleen with Serr. marcescens endotoxin produced no significant change in the tumor-damaging potency of this polysaccharide. However, 40% homogenates of these organs altered this endotoxin so that it no longer elicited hemorrhagic-necrosis in Sarcoma 37. Experimental work was thereafter restricted to rabbit liver which was freed of blood by thorough perfusion. Liver homogenates were fractionated by standard cytochemical and centrifugal procedures and it was found that neither nuclei nor mitochondria possessed endotoxin-detoxifying activity. The supernatant which remained was active; but this activity was lost when the microsomes were removed. However, combination of microsomes and supernatant restored endotoxin-inactivating potency. Preliminary experiments on the experimental conditions of test which influence the inactivation of endotoxin by this tissue preparation indicate that it may be different from the EDC in blood.

4. An investigation was begun of the effects of selected carcinogens on natural immunity of the mouse. It has been demonstrated by other investigators that large doses of selected carcinogens (methylcholanthrene, benzopyrene, and others) produce marked depletion of host lymphoid tissue. In addition, there is suppression of bone marrow activity and hemorrhagic necrosis of the small intestine in mice receiving large doses of carcinogens. Because the tissue damage produced by carcinogens resembles the tissue destruction that results from total body irradiation, it seems reasonable to assume that the tissue damage produced by carcinogens likewise is associated with profound alterations in host resistance. It is also likely that these alterations in host resistance would play a significant part in the pathogenesis of tumors resulting from these carcinogens.

Our work thus far has been primarily concerned with determining the toxicity of methylcholanthrene for the CAF_1 mouse. The observations of others have been confirmed and it has been determined that 0.1 gm/kg. of methylcholanthrene is sufficient to produce maximum tissue damage without lethality. In addition, it has been found that the hematocrit and the clearance by the R.E.S. of a standard dose of colloidal carbon injected i.v. are the most sensitive indicators of lymphoid and bone marrow depletion.

5. In last year's annual report we described the characteristics of a substance in serum designated EDC (endotoxin detoxifying component) and the experimental conditions under which it altered isolated endotoxins in vitro so that they no longer were capable of eliciting endotoxic or antigenic responses in the mammalian host. These studies have been extended to ascertain whether endotoxin in its native state on the bacterial surface was likewise susceptible to inactivation by an agent with the distinctive features of EDC. Two characteristic attributes of the endotoxin of intact Salmonella typhosa, the induction of hemorrhagic necrosis in Sarcoma 37 and the stimulation of specific antibody formation in rabbits were the test situations employed to measure changes in the endotoxin of typhoid bacilli following interaction in vitro with serum. The endotoxic and antigenic activities of typhoid bacilli killed by heat or by chemical treatment were abolished by EDC, the same agent previously shown to inactivate isolated endotoxin. In contrast, the endotoxic and antigenic activities of viable bacilli were unaffected by EDC; they were, however, eliminated by a different system in fresh serum. This inactivation, of the endotoxic properties of viable typhoid bacilli, by the sera of various animal species was found to parallel their bactericidal activity for S. typhosa. It was shown that the same components in serum were required for both effects, viz., normal antibody, all four of the recognized components of complement and divalent cations. Serum rendered deficient in any one of these six constituents of the system no longer was capable of eliminating endotoxic potency or exerting the bactericidal effect. The quantities of serum required, and the kinetics of the interaction in vitro and in

certain in vivo situations, were strikingly similar for both effects, attesting to the intimate association of these changes effected in the bacilli by serum. These changes appear to be simultaneous events; it has not been possible to effect a separation of the killing of the bacilli by serum from the loss of their toxic and antigenic properties. However, the essentiality or inevitability of these changes for the bactericidal process itself remains to be determined. Which of the two humoral systems in normal serum inactivates the endotoxin of typhoid bacilli depends on whether the organisms are viable or not. The concomitant elimination of endotoxic attributes of typhoid bacilli in the bactericidal process of normal serum has obvious important implications for the host-parasite relationship.

6. The plasma of febrile rabbits that have been treated with bacterial lipopolysaccharides has been reported to contain "endogenous pyrogen", a substance with fever producing capabilities quite different from those of the originally injected polysaccharide. This led to the hypothesis that endogenous pyrogen is a product of tissue injury produced by the injected bacterial endotoxin. However, an equally plausible hypothesis which we are exploring is that endogenous pyrogen is the injected endotoxin which has been altered by the host cells and plasma. Reduction of the pyrogenicity of endotoxin following incubation in vitro with normal human serum was first reported several years ago. Studies in this Laboratory have since demonstrated that detoxification of endotoxin by human plasma is not limited to pyrogenicity and that alterations in other biologic properties of endotoxin are equally impressive. These observations lend strong support to the contention that endotoxin can be profoundly altered by the host's plasma and that endogenous pyrogen may, indeed, be altered endotoxin.

Experiments were designed to study in detail the pyrogenic properties of endotoxin that had been altered in vitro by incubation with human plasma. The following preliminary data are available: 1) Altered endotoxin produces fever in endotoxin-tolerant animals;

2) There is no leukopenia associated with the pyrogenic response; 3) Animals do not become tolerant to daily injections of altered endotoxin; 4) There is no acute refractoriness to altered endotoxin when multiple injections are given over a short period of time. The above properties of endotoxin altered by plasma have been the very ones used to distinguish endogenous pyrogen from bacterial endotoxin. These data are therefore interpreted as providing support for the hypothesis that endogenous pyrogen is endotoxin which has been altered by the host's plasma.

7. Adrenalectomized mice are known to be unusually susceptible to the lethal properties of bacterial lipopolysaccharides. Treatment with 400-500 micrograms of endotoxin are required to kill normal mice; on the other hand, only 2-5 micrograms are necessary to kill adrenalectomized mice. It was, therefore, visualized that this specially prepared animal might provide a sensitive and generally available test animal for the assay of endotoxin and for the endotoxin detoxifying component in plasma.

It was found that the detoxification of endotoxin by EDC could indeed be shown in the adrenalectomized mouse. However, the quantity of this factor required was greater when tested in the adrenalectomized mouse than when the reaction mixture was tested for other biologic properties of endotoxin. Consequently, the adrenalectomized mouse has but limited usefulness for assaying EDC since only plasmas containing large amounts of the factor could be readily assayed by this procedure.

8. The bactericidal system of normal and immune sera, in which antibody and complement are the principal participants, causes the death or lysis of Gram-negative bacteria. The susceptibility of individual strains of these organisms to the lethal effect of serum varies widely. The means by which fresh serum evokes this cidal effect is not known, nor is information available to account for differences in sensitivity to serum among various species and strains of Gram-negative bacteria. It has been reported that the endotoxins of these bacteria

(somatic antigens, lipopolysaccharides) interfere with the bactericidal effect of serum in vitro. We have sought to determine whether susceptibility to serum of antigenically identical or closely related strains was correlated with differences in their content of endotoxin and/or somatic antigen. Two such closely related strains of Escherichia coli were isolated from a single clone and were shown to differ markedly in their sensitivity to serum. The supernatant fluids of boiled suspensions of these organisms served as antigen preparations which were readily adsorbed on erythrocytes. Such antigen-coated erythrocytes were agglutinated by specific antibody. The extracts from the serum resistant strain contained much more of the cell sensitising antigen than did the preparation from the sensitive strain. It was found that acetone-killed and dried cells of the resistant strain, as well as extracts of this culture, were considerably more potent antigens for rabbits than their counterparts from the serum susceptible strain. Furthermore the cells and the extracts of the resistant strain exhibited markedly greater endotoxic potency (induction of hemorrhagic necrosis in Sarcoma 37) than analogous products from the sensitive strain. These findings indicate that the quantity of somatic polysaccharide complex on these bacteria may determine their susceptibility to the bactericidal system of fresh serum.

The occurrence in normal serum of antibodies specific for bacteria of several genera of Enterobacteriaceae (25 strains of Escherichia, Salmonella and Shigella were examined) was investigated. It was found that antibodies reactive with each of these strains were present in normal human and rabbit sera. These natural antibodies are present in low concentration, but the quantities are sufficient to permit maximal expression of the bactericidal action of these sera. The bactericidal action of normal serum against small inocula of E. coli was not increased by the addition of specific immune antibody.

9. The findings in studies on the effects of normal serum on the endotoxic polysaccharides of Gram-negative bacilli and on their viability, presented in another section of this report, were utilized in a search for humoral systems which might affect tumor cells. Exploratory experiments revealed that serum exerted an impressive lethal

effect on tumor cells. Incubation of Sarcoma 37 ascites cells with normal human serum abolished their capacity to proliferate in mice. This lethal effect of serum on tumor cells was investigated systematically and it was found that this activity was exhibited by a system distinctive by virtue of its species distribution, the many components required for its action, and the range of mouse tumors it affected. Most of the experimental work was carried out with cells of Sarcoma 37 in the ascitic form and with fresh normal human serum. Cells and serum were interacted in vitro for 1 hour at 37°C., in a ratio of $1x10^6$ cells per ml. of serum, and the effects on the cells were ascertained in four ways. a) Failure of the cells to proliferate in vivo. b) Loss of metabolic activity, with glucose as substrate, as measured in the Warburg respirometer (in collaboration with Dr. N. W. Woods), or by the reduction of neotetrazolium. c) Uptake of eosin as evidence of cell death. d) Morphologic changes, notably the extensive blebbing of the cell membrane. These four parameters yielded consistent results in the assessment of cell viability. The cytotoxic activity of serum was eliminated by heating at 52°C. for 1/2 hour, or by absorption with washed sarcoma cells at 4°C. The original cytotoxic activity was restored on combining the heated serum with the absorbed one, an indication that at least two factors were required. Inasmuch as such heat treatment inactivates C'2, and since the absorbed serum was found to retain its hemolytic activity, it appeared that complement might be involved. Tests with the standard reagents R1, R2, R3 and R4 prepared from human serum showed that absence of any one of the four recognized components of complement resulted in loss of cytotoxic activity. Appropriate combination of these reagents restored activity. Addition of EDTA to serum resulted in loss of cytotoxicity; restoration of activity was accomplished by the addition of Ca^{++} or Mg^{++}; thus, divalent cation was also essential for the reaction. The reaction between human serum and the tumor cells was explored as regards serum concentration, number of cells, temperature, and incubation time. Serum diluted 1:8 showed undiminished activity while a 1:16 dilution produced a partial effect. One ml. of serum destroyed the proliferative capacity of an inoculin as large as $3x10^6$ cells and exerted a considerable, but incomplete effect on $7x10^6$ cells. The reaction proceeded optimally at 37°C.,

with a lethal effect exerted within 2 minutes. One-half hour was required for cytotoxic action at 20°C., but at 4°C. cell viability remained unaffected.

The cytotoxic effect obtained with human serum was also exhibited by sera from chimpanzee, rhesus monkey, cow, pig, dog, cat, rabbit, chicken and goose. Non-cytotoxic sera were those from horse, sheep, goat, guinea pig, hamster, rat and mouse. The factor absorbed from human serum by Sarcoma 37 cells could also be removed by other mouse ascites tumors including the Ehrlich and Krebs carcinomas, hepatomas 129 and 134 and Sarcoma 180 as well as by cell suspensions prepared from solid tumors of Sarcoma 37 and Melanoma S-91. Suspensions of normal mouse cells of liver, lung, kidney, heart, muscles and erythrocytes effected little or no absorption of this factor, but spleen absorbed it completely. Preliminary experiments on the conditions for adsorption of the factor on tumor cells, its elution from these cells, its heat lability and other properties indicate that it behaves like a normal antibody reactive with receptors on these mouse tumor cells. Sera injected into mice i.p. immediately prior to or following i.p. challenge with Sarcoma 37 ascites failed to exert a cytotoxic effect *in vivo*.

There is thus present in the normal serum of a number of species a factor resembling antibody which, together with complement exerts a lethal effect on mouse tumor cells *in vitro*. The action of normal serum on these cells bears a striking resemblance to the effects produced by hyperimmune serum.

10. Human serum has been shown by Dr. B. Björklund of the State Bacteriological Laboratory, Stockholm, Sweden, to contain a cytolytic factor coupled to an inhibitor. The factor was dissociated from its inhibitor by dialysis and obtained in a relatively pure state following electrophoresis. In the isolated state it displayed a lytic effect on four established cell strains of human origin. In collaboration with Dr. Björklund, exploratory experiments were carried out to examine the effect on mouse tumor cells exerted by this factor (CF) and other components separated from normal human serum by paper curtain

electrophoresis. CF produced marked morphologic changes in Sarcoma 37, Krebs, Ehrlich and other ascites tumor cells manifested by multiple lesions in the cell membrane. Following this in vitro interaction the S-37 cells no longer proliferated in mice. The cytotoxic effect produced by this heat stable factor was manifestly different from that of the antibody-complement system described in another section of this report.

Significance to the Program of the Cancer Institute:

There is present in the normal serum of many species a system of normal antibody and complement which is lethal for various mouse tumor cells in vitro. The effects exerted on tumor cells duplicate those reported for hyperimmune serum. Furthermore heat stable fractions have been isolated from normal serum which are also cytotoxic for tumor cells. These findings offer tangible evidence that the host may possess normal components which influence the growth and behavior of atypical cells and thereby contribute to natural resistance to neoplasia.

Proposed Course of Project:

1. The following parameters of natural immunity will be measured in mice that have been treated with toxic, carcinogenic doses of methylcholanthrene and benzopyrene:

   a. Survival of an heterologous skin graft.
   b. Resistance to a standardized skin infection.
   c. Antibody response to a well-defined antigenic stimulus.
   d. Reaction to the toxic and lethal properties of bacterial polysaccharides.

2. Investigation of the endotoxin detoxifying component in plasma will be continued with the objective of elucidating the relationship between endogenous pyrogen and plasma-reacted endotoxin. To obtain such information attempts will be made to prepare animals whose plasma is deficient in EDC. Medical and surgical extirpation experiments will be carried out, including the following: (1) treatment with nitrogen mustard; (2) hepatectomy; (3) pancreatectomy; (4) splenectomy; (5) and nephrectomy. Such animals will be tested, in addition, for their ability to produce endogenous pyrogen.

3. Work on the purification of the endotoxin detoxifying system in mammalian tissues will be continued in collaboration with Dr. Waravdekar, Armed Forces Institute of Pathology. The relationship of this system to known enzymatic activities of these tissue preparations will be examined. The effects of various experimentally induced pathologic states on this tissue activity in rabbit liver will be studied.

4. A number of additional strains and species of Gram-negative bacterial genera will be examined to determine whether the correlation between susceptibility to the bactericidal action of normal serum and content of somatic antigen and/or endotoxin, as found in E. coli, represents a general phenomenon.

5. Studies will be initiated to compare the participation of natural vs. immune antibody in the bactericidal process as regards the following:

a. Their effectiveness for sensitizing bacteria to the lethal action of complement.
b. Their relative specificities as revealed by quantitative absorption tests with homologous and heterologous microorganisms.

6. Study of the complement-dependent system of normal serum lethal to mouse tumor cells will be continued with the following objectives:

a. The component of the system which resembles natural antibody will be concentrated and its properties examined in greater detail. Information will be sought to account for its bizarre distribution in animal species.
b. The range of tumor cells affected by this system will be ascertained.
c. Experiments will be designed to account for the lack of serum cytotoxicity in vivo.
d. In collaboration with Dr. M. W. Woods, Lab. of Biochemistry, NCI, the mechanism of the injury inflicted on the tumor cells will be investigated with special attention to the striking effects on glycolysis.

7. The findings with serum fractions (prepared by Dr. Bjorklund) have made it evident that in normal human serum at least two separate components are present which, in the isolated state, are capable of exerting strong cytotoxic effect on mouse tumor cells. It is proposed to examine the conditions under which these effects are exerted and to determine the extent to which individually or collectively they contribute to the cytotoxic effect of normal serum.

Part B included --- Yes.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) Landy, M., Trapani, R-J., and Shear, M. J.: Inactivation of endotoxin by a humoral component. IV. Alteration in the immunological properties of typhoid endotoxin. J. Exper. Med. 110: 731-750 (1959).

(2) Rosen, F. R., and Landy, M.: Inactivation of endotoxin by a humoral component. V. EDC activity during Gram-negative infection and shock. Zeit. f. Immunitäts-forsch. 118: October (1959).

(3) Landy, M., Trapani, R-J., and Rosen, F. S.: Inactivation of endotoxin by a humoral component. VI. Two separate systems required for viable and killed Salmonella typhosa. J. Clin. Investig. In press 39: (February) 1960.

Honors and awards relating to this project:

The Head of the Polysaccharide Section was invited to participate in an International Conference on Biological Effects of Endotoxins in Relation to Immunity held in Freiburg, Germany, July 27-31, 1959.

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1. Endocrinology Br
2. Endocrinology Serv
3. Bethesda, Md.

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Part A.

Project Title: Endocrine Aspects of the Progression and Therapy of Cancer of the Breast in Women and Men.

Principal Investigators: Dr. D. M. Bergenstal, Dr. R. Hertz and Dr. M. B. Lipsett

Other Investigators: Dr. S. M. Kahn, Dr. T. L. Goodfriend, Dr. D. A. Kellogg, Dr. R. H. Moy, Dr. H. Schwartz, Dr. S. Genuth, Dr. J. Lewis, Mr. S. Jackson.

Cooperating Unit: (Dr. J. Van Buren)

Man Years (calendar year 1959):
Total: 16
Professional: 11
Other: 5

Patient Days (calendar year 1959)
Patients
Days

Project Description:

Objectives: 1. To extend and intensify detailed clinical observation of the natural course of cancer of the breast in men and women in order to better appraise the effectiveness of various methods of therapy and learn more concerning the etiology and pathogenesis of the disease.

2. To improve upon existing forms of hormonal therapy for palliation of advanced breast cancer and to elucidate the mechanisms involved.

Methods Employed: 1. The acceptance of complete clinical responsibility for the further management of referred patients with proven diagnosis of cancer of the breast and the application to their problems of all available modes of therapy.

2. The exploration of new modes of hormonal therapy for palliation.

3. The detailed analysis of endocrine and metabolic factors operating in relation to the genesis and course of the disease in these patients up to the time of their demise.

4. Careful appraisal of pathological specimens obtained at biopsy or autopsy in collaboration with Pathology staff.

Major Findings: Metabolic effects of growth hormone:

1. Extensive metabolic balance studies with chymotrypsinized beef growth hormone, whale growth hormone, and sheep growth hormone have revealed these products to be inactive in man. Human growth hormone was shown to be an effective anabolic agent in a variety of clinical studies. A significant effect of human growth hormone was an increase in urinary calcium excretion from 20 to 150 mg. daily.

In an attempt to demonstrate the possible role of growth hormone in tumor growth, we have administered human growth hormone to ten patients with metastatic breast cancer, three patients with metastatic adrenal cancer, and two patients with metastatic carcinoma of the prostate. In no instance was there clinical exacerbation of the disease. The measurable parameters of tumor activity in the last two groups were unchanged. From the previous studies, it is apparent that an increase in urinary calcium following the administration of growth hormone is an inadequate criterion of tumor growth. In the patients undergoing a remission following hypophysectomy, the urinary calcium was unchanged following growth hormone. Only in those patients in whom there was evidence of tumor progression did growth hormone produce some rise in the urinary calcium. Thus, for these short periods of administration, we could find no evidence to suggest that human growth hormone facilitates or stimulates tumor activity.

Therapy of cancer of the breast in human.

Hypophyseal stalk section. In collaboration with Dr. J. Van Buren of NINDB, we have performed hypophyseal stalk section in 11 patients with metastatic breast cancer. This was done with the expectation that the procedure would be simpler, thus decreasing the morbidity. However, for reasons which are not apparent at this time, the morbidity in this group was considerably higher than in our hypophysectomy group. Furthermore, the results of treatment have not been as good as those following hypophysectomy. It has, therefore, been decided to terminate this study at this time, and to intensively follow those patients who had been subjected to stalk section.

The data with respect to endocrine function following stalk section revealed pituitary-endocrine relationships not heretofore described. Adrenal function almost uniformly decreased to hypophysectomy levels. Gonadotropin excretion was variable for many months after surgery. Four patients have demonstrated normal thyroid function that could be suppressed by the administration of triiodothyronine. From these data, several tentative conclusions may be drawn:

Major Findings (con't.):

1. Following hypophyseal stalk section, there is variable destruction of the anterior pituitary gland.

2. Contrary to studies in the dog, ACTH production appeared to be the most sensitive indicator of pituitary gland destruction.

3. In the absence of hypothalamic-pituitary vascular connections, the thyroid-pituitary axis was normal in several patients and gonadotropin production continued.

Hypophysectomy.

Though anterior hypophysectomy has been continued in favorable patients for the purpose of having these patients available for study, the therapeutic results do not differ from those previously reported.

2-Deoxyglucose.

The studies on the effects of 2-deoxyglucose on breast cancer have been brought to a close. Inconclusive data suggest that suppression of the metabolic activity of breast cancer as manifested by reduced calcium excretion can be observed in properly selected cases. These observations are submitted for publication by Dr. S. Kahn and Dr. D. M. Bergenstal.

Significance to the Program of the Institute: The direct pertinence of the foregoing studies to the grave problem of advanced or persistent breast cancer is too obvious to require discussion.

Proposed Course of Project: The further course of these studies are to be essentially along the lines outlined above. Emphasis is to be placed on balance studies and the evaluation of hypophysectomy in breast cancer. Further evaluation of new steroids and related compounds is to be made.

Part B included.

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Part B. Honors, Awards, and Publications

Honors and Awards relating to this project:

Dr. D. M. Bergenstal

Co-chairman, American College of Physician 6th Postgraduate course on Endocrinology.

Consultant to Cooperative Group Study on Lung Cancer.

Presented a paper at the meetings of The Association of American Physicians, Atlantic City, N. J., May 1959.

Dr. Roy Hertz

Invited to lecture at Academy of Medicine of Cleveland and Cuyahoga County Medical Society, Cleveland, Ohio, November 19, 1959.

Participated in semi-annual meeting of The Medical and Chirurgical Faculty of the State of Maryland, Ocean City, Md., September 18, 1959.

Moderator on panel of the 45th Annual Clinical Congress of the American College of Surgeons, Atlantic City, N. J., September 28, 1959.

Participated in meeting of the Brooklyn Academy of Pediatrics held at Bethesda, Md., May 20, 1959.

Chairman of the Endocrinology Section of the American Association for Cancer Research at their 1959 meeting, Atlantic City, N. J., April 11, 1959.

Invited speaker of The Eastern Conference of Radiologists, Washington, D. C., March 1959.

Dr. M. B. Lipsett

Lectured before the House Staff of Georgetown University Medical School; Howard University Medical School; Washington Hospital Center, and Washington Sanitarium.

Invited speaker at Piedmont Postgraduate Medical School, Clemson, S. C.

Member, Faculty of Postgraduate course, American College of Physicians, Bethesda, Md., Feb. 1959.

Serial No. NCI-803(C)
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Nature of Hormone-Producing Tumors of Pituitary, Adrenal, Ovary, Testis, Pancreas, Parathyroid and Chorion; Abnormalities of Somatic Development and Growth.

Principal Investigators: Dr. Roy Hertz, Dr. D. M. Bergenstal and Dr. M. B. Lipsett

Other Investigators: Dr. R. H. Moy, Dr. John Lewis, Dr. S. Genuth, Dr. D. A. Kellogg, Dr. T. L. Goodfriend, Dr. H. Schwartz, Mr. H. L. Erwin, Mr. S. Jackson and Mr. P. Rayford

Cooperating Units: Dr. R. Bates and Dr. H. Wilson, NIAMD; Dr. L. Garren, Pharmacology Branch, NCI.

Man Years (calendar year 1959):
Total: 16
Professional: 11
Other: 5

Patient Days (calendar year 1959)
Patients
Days

Project Description:

Objectives: 1. To learn more about the basic metabolic phenomena involved in the process whereby neoplasms in hormone-producing organs produce highly excessive amounts of hormone, resulting in such syndromes as Cushing's disease, acromegaly, hyperparathyroidism, hyperadrenalism, and similar endocrinopathies.

2. To maintain in tissue culture and in animals hormone-producing tissues and to ascertain the factors involved in quantitative hormone production in vitro.

3. To assess the capacity of new drugs to alter the course and hormonal activity of hormone-producing tumors.

Methods Employed: Hormone producing tumors from patients with one of the above-named syndromes are obtained at surgery and carried in tissue culture in irradiated, cortisonized rats and hamsters. The supernatant fluid from the cultures are tested for hormonal activity by appropriate bioassay methods and the quantity recovered compared with the content of a piece of tissue exactly equivalent to that originally placed in culture.

Patients presenting such syndromes are completely characterized endocrinologically and metabolically before and after therapy in order to ascertain the relative effects of various forms of therapy.

Major Findings:

1. Studies on Choriocarcinoma.

(a) Fifty patients with choriocarcinoma or related metastatic trophoblastic disease have now been studied from both a therapeutic and more basic aspect. Twenty-one of 44 patients who were admitted more than 3 months ago show no residual evidence of disease for periods ranging from 5 to 47 months. Eight patients are now resistent to methotrexate with persistent disease as evidenced by continuing hormone titer. Fifteen patients have died following initial response with subsequent development of drug resistance. These data provide a very adequate statistical basis for the evaluation of methotrexate therapy in advanced metastatic-trophoblastic disease, indicating that almost half such patients can be effectively freed of evidence of residual disease.

(b) The problem of the methotrexate resistant patient remains our most pressing concern. Repeated therapeutic attempts with nitrogen mustard, cytoxan, and actinomycin have yielded no significant responses.

Since May, 1959 we have treated 8 methotrexate resistant choriocarinoma patients with Vincaleukoblastine, an alkaloid derived from the periwinkle plant (Vinca rosea) (Noble, Cutts and Beer, 1957). (See also project 808 for our animal studies indicating the sensitivity of heterologously transplanted choriocarcinoma to this agent).

Three of these patients now have normal gonadotropin titers, suggesting their complete remission, the longest being 5 months in duration. Two additional patients showed definite but rapidly reversible evidence of tumor regression. The remaining three patients showed no apparent response to the drug. Thus we appear to have at least some recourse against methotrexate resistance and possibly a highly potent agent for primary therapy. Limitations in available supply of the drug have retarded the progress of our studies.

Reversible toxic effects of Vincaleukoblastine have included: (a) bone marrow depression, (b) alopecia, (c) neurotoxicity, (d) stomatitis, (e) malaise and depression, and (f) local phlebitis.

The most significant aspect of these findings is the fact that Vincaleukoblastine is chemically unrelated to any previously used oncolytic agent, thus opening a large area for investigation in the alkaloid field. These studies are submitted to Cancer Research for publication.

Major Findings (con't.)

(c) The problem of the immune relationship between choriocarcinoma and the host subject has been frequently raised since this tumor is derived from the zygote which contains both paternal and maternal genes. We have therefore undertaken a study of the blood groups of women with choriocarcinoma in relation to their husbands' blood groups. These studies indicate no evidence of cross-immunization between husband and wife even in those patients whose tissues are extensively invaded by tumor. It is inferred that this embryonic tissue lacks significant antigenicity for the host at least as far as circulating anti-body formation is concerned. Further studies regarding fixed tissue antigens are in progress. These observations are being submitted for publication in the American Journal of Obstetrics and Gynecology in collaboration with Dr. Paul Schmidt of our Blood Bank.

(d) Normal placenta has been shown to contain two types of steroid responsive enzymes - an estrogen activated dehydrogenase and a steroid transhydrogenase. These enzymes have been demonstrated in very small amounts in choriocarcinoma tissue and the further characterization of these enzymes as well as their histochemical localization in tumor as well as placental tissue will be of great interest (Dr. Donald A. Kellogg).

(e) Methotrexate has been found to completely inhibit the transformation of phenylalanine to tyrosine. This interference is consistently observed in the methotrexate treated patient and may play a vital role in the mechanism of action of the folic antagonist (Dr. Theodore Goodfriend).

During the past year, we have treated 14 patients with metastatic adrenal cancer with o,p' DDD. Five patients have shown objective regression of cancer. There have been 7 deaths in the group. All patients with functioning adrenal cancer have shown some suppression of steroid output with the drug.

We are starting to correlate plasma levels of o,p' DDD with dosage and response. Preliminary observations suggest that some of the poor responses to o,p' DDD occurred in spite of adequate absorption of the drug.

In collaboration with Dr. William Tullner, we have initiated a study regarding the disappearance rate of o,p' DDD from plasma. This will be examined by both standard chemical techniques and the use of tritiated o,p' DDD.

In 2 of the patients whose ketosteroids had reached normal levels, paper chromatography revealed that a large proportion of these was dehydroepiandrosterone. This suggests continuing low-grade activity

Major Findings (con't.)

of the cancer, even though a significant remission was obtained.

Studies in Steroid Metabolism.

1. In collaboration with Dr. Wilson of NIAMD, we have initiated a study of the urinary metabolites of important precursors of hydrocortisone and dehydroepiandrosterone. From this study, it should be possible to delineate the areas of enzymatic blocks in a variety of types of adrenal cancer. Due to the very large excretion of all types of steroids in these patients, considerable purification has to be performed in order to accurately measure any single metabolite.

2. In last year's report, we outlined the project to study the initial stages of stimulation of the adrenal cortex with ACTH, in collaboration with Dr. Wilson. This has been completed and we have shown that the biosynthesis of adrenal androgens is less dependent on ACTH than is the synthesis of cortisol. These results are being reported in detail in the Journal of Clinical Endocrinology and Metabolism.

3. The study of the precursors of dehydroepiandrosterone in collaboration with Dr. Wilson has proved difficult because of the problems encountered in separating one of the metabolites. We are still actively pursuing this, however.

4. A study has been initiated to determine the proportion of $C_{19}O_2$ metabolites in the urine arising from dehydroepiandrosterone. Tritiated dehydroepiandrosterone has been given to ovariectomized patients, as well as patients with adrenal cancer, and the specific activities of the urinary $C_{19}O_2$ metabolites measured. This will yield information about the role of dehydroepiandrosterone as the precursor of the androgenic metabolites excreted in the urine.

5. The previously described study of women with hirsutism has been completed and has been accepted for publication in the Journal of Clinical Metabolism. We were unable to correlate any of the $C_{19}O_2$ or $C_{19}O_3$ metabolites or pregnanetriol with either the extent or severity of the hirsutism.

6. The availability of orally effective progestational compounds free of estrogen activity permitted an examination of the effects of high doses of progestin. In contrast to progesterone, Provera caused a negative nitrogen balance only once in 7 attempts. It did not have any anti-aldosterone activity. Provera failed to suppress gonadotropin excretion in post-menopausal patients and in

Major Findings (con't.)

3 girls with precocious puberty. Partial characterisation of urinary metabolites of Provera was undertaken because of the finding that the administration of this compound resulted in large increases in the excretion of Porter-Silber chromogens. Although the structure of the functional group was not determined, known functional groups were ruled out as a source of this reaction. This study will not be pursued, although there is, undoubtedly, a previously unrecognized phenylhydrazine-reacting group in metabolites of Provera. These studies are being reported in the American Journal of Obstetrics and Gynecology.

7. An amphenone derivative which has the capacity to block 11β-hydroxylation has been studied, and a test for pituitary ACTH reserve standardized. This will prove useful clinically in evaluating patients with pituitary tumors.

8. In collaboration with Dr. Leonard Garran of the Pharmacology Branch, NCI, we have examined the influence of non-thyroidal hypercatabolic states upon the metabolism of cortisol. The disappearance rate of 4-C^{14}-Cortisol and the secretion rate of cortisol have been measured in patients with leukemia before and after correction of the hypermetabolic state. No differences have been observed. This study will be expanded to include the effect of fever induced by pyrogens on the metabolism of cortisol.

Metabolism.

1. In the patients with panhypopituitarism, low doses of cortisol and triiodothyronine were shown to have significant catabolic effects. The influence of the endocrine status of the patient with respect to the catabolic effects of cortisol had not previously been examined. From our studies, it is apparent that physiological effects of these hormones cannot be compared unless the endocrine status of the subject is well-defined.

These studies have been submitted to Metabolism.

2. Glucagon was shown to produce a rise in plasma unesterified fatty acids 48 hours following administration. Its catabolic effect has been studied and demonstrated to be independent of hepatic glycogenolysis. These studies have been submitted to the Journal of Laboratory and Clinical Medicine.

3. A congener of testosterone, testololactone, was shown to be metabolically inert in 2 balance studies. It did, however, markedly reduce the hypercalciuria accompanying osteolytic bone disease due to carcinoma of the breast. There have been no objective regressions of disease.

Major Findings (con't.)

The effects of growth hormone have been examined in 3 patients with active acromegaly. No nitrogen retention was observed, although the urinary calcium excretion increased. These same patients demonstrated an ability to retain nitrogen when testosterone was given. This phenomenon has not previously been noted, and implies either different sites of deposits of protein or different mechanisms for nitrogen anabolism. In collaboration with Dr. Julius White, of the Laboratory of Physiology, we have begun an investigation into certain parameters of protein metabolism using N^{15}-labeled glycine. We intend to study patients with active acromegaly before and after treatment. N^{15}-glycine will also be given to patients on metabolic balance during periods of administration of human growth hormone and testosterone, in an effort to delineate the possible different mechanisms of nitrogen retention.

Significance to the Program of the Institute: Further understanding of the conditions permitting the excessive hormonal production of tumors of endocrine origin is basic to our understanding of the metabolism of neoplastic tissue generally. In addition, further analysis of abnormalities in somatic growth and development should provide data pertinent to the problems of tissue growth generally.

Proposed Course of Project: Further elaboration of these studies with major emphasis on biochemical and pharmacological control of hormone-producing tumors and anomalous tissue growth is projected.

Part B included.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Lipsett, M. B. and Bergenstal, D. M.: The Metabolic and Corticotrophin-Suppressing Activity of Certain C_{21}-Deoxy-steroids. Acta Endocrinol. 31: 33-39, 1959.

2. Lipsett, M. B. and Bergenstal, D. M.: Metabolic Effects of Amethopterin in Man. Cancer Research 19: 89-92, 1959.

3. Engel, H. R., Bergenstal, D. M., Nixon, W. E. and Patten, J. A.: Effect of Human Growth Hormone on UFA and Plasma Amino Acid Nitrogen in Man. Proc. Soc. Exper. Biol. & Med. 100: 699-701, 1959.

4. Lubs, H. A., Vilar, O. and Bergenstal, D. M.: Familial Male Pseudohermaphrodism with Labial Testes and Partial Feminization: Endocrine Studies and Genetic Aspects. J. Clin. Endocrinol. & Metab. 19: 1110-1120, 1959.

5. Bergenstal, D. M., Lipsett, M. B., May, R. H. and Hertz, R.: Regression of Adrenal Cancer and Suppression of Adrenal Function in Man by o,p' DDD. Trans. Assoc. Amer. Phys. 72: 361- 1959.

6. Lipsett, M. B. and Bergenstal, D. M.: The Catabolic Effects of Low Doses of Cortisol and Triiodothyronine in the Panhypopituitary Subject. Metabolism (in press).

7. Lipsett, M. B., Engel, H. R. and Bergenstal, D. M.: Effect of Glucagon on Plasma Unesterified Fatty Acids and in Nitrogen Metabolism. J. Lab. & Clin. Med. (in press).

8. Lipsett, M. B. and Riter, B. D.: Urinary Ketosteroids and Pregnanetriol in Hirsutism. J. Clin. Endocrinol. & Metab. (in press).

9. Hertz, R.: Gonadotropin and Adrenocorticotropin from Rat Pituitary Homografts as Manifested by Host Response to Chorionic Gonadotropin and Amphenone. Endocrinology (in press).

Part B. (con't.)

Honors and Awards Relating to this Project:

Dr. Roy Hertz

Re-appointed to serve on the Postgraduate Committee of the Endocrine Society during 1959-60.

Member, Awards Committee of the Endocrine Society.

Participated as guest lecturer, Squibb Centennial Lecture Program, February 1959.

Invited to lecture at Mayo Clinic, Rochester, Minnesota, April 27, 1959.

Guest speaker, Minnesota State Gynecological and Obstetrical Society, Duluth, Minn., April 25, 1959.

Lectured at the University of Southern California Medical School, April 23, 1959.

Participated in meeting of the New York Cancer Society, April 14, 1959.

Chairman, meeting of the Mick Carter Club, Bethesda, Md., November 6, 1959.

Participated in seminar of Armed Forces Institute of Pathology, December 1, 1959.

Dr. D. M. Bergenstal

Invited to participate in conference on The Biological Activities of Steroids in Relation to Cancer, CCNSC, Vergennes, Vt., Sept. 1959.

Dr. M. B. Lipsett

Participated in meeting of the Mick Carter Club, Bethesda, Md., November 6, 1959.

Participated in Brook Lodge Conference on progestational agents.

Invited speaker at Postgraduate Course, Freedmen's Hospital, Washington, D. C.

Serial No. NCI-808(C)
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Endocrine Factors Governing Hormone-Induced Tissue Growth and Tumor Formation in Animals; Bioassay of Blood and Urine from Cancer Patients

Principal Investigator: Dr. Roy Hertz

Other Investigators: Dr. W. W. Tullner, Dr. J. Lewis, Dr. H. Schwartz, Dr. S. Genuth and Mr. H. Erwin

Man Years (calendar year 1959):
Total: 9
Professional: 6
Other: 3

Patient Days (calendar year 1959)
Patients
Days

Project Description:

Objectives: 1. To gain basic information concerning endocrinological and nutritional factors governing hormonally conditioned growth and involution in organs such as the uterus, breast and prostate which are the frequent seat of hormone-sensitive neoplasms.

2. To develop and improve bioassay methods for the quantitation of hormones in the blood and urine of patients with cancer who are either receiving various forms of hormonal therapy or who present unusual hormonal patterns.

3. To determine the biological properties of steroid and related compounds in terms of their potential usefulness in cancer therapy.

Methods Employed: Standard methods of biological study are employed. These include:

1. The maintenance of animals of various species on specified hormonal or nutritional regimens.

2. Daily observations of physical and behavioral changes in such animals.

3. Complete autopsy of test animals with detailed weighing and chemical analysis and histochemical study of specific organs.

4. Surgical alteration of the endocrine status of animals by gonadectomy, adrenalectomy, hypophysectomy, thyroidectomy, parabiosis, hysterectomy and other operative procedures.

Major Findings: 1. (a) Four strains of human choriocarcinoma have now been established as heterologous transplants in the hamster cheek-pouch. Two of these strains no longer require prior cortisone administration to the hamster. Each of these tumors grows rapidly for 2 to 3 weeks and then undergoes necrosis, liquefaction and total resorption during the ensuing week or two. Continued cortisone injection does not protect the tumor from the host reaction. Histologically the tumor tissue contains both syncytiotrophoblast and cytotrophoblast. The tumor cells are highly pleomorphic. The tumor tissue is richly vascularized and one may estimate that at least half of the tissue mass is made up of newly proliferated endothelial channels filled with blood cells which are clearly of host origin. There is abundant mitotic activity but in no instance have metastases or even invasion through the cheek-pouch been observed.

(b) The endocrine properties of the transplantable choriocarcinomas are very striking. The tumor is productive of large amounts of chorionic gonadotrophins. This is readily detectable in the tumor tissue, in the plasma and in the urine of the hamster. However, it is undetectable in the animal's peripheral tissues such as the liver, kidney, spleen or gonads. It would appear that the chorionic hormone has sufficient molecular size to be excluded from entry into peripheral tissue cells but can still be filtered through the glomeruli of the kidney. This suggests some selective handling by the kidney. Moreover, the absence of demonstrable hormone from the gonads, even though the latter show marked trophic response to the hormone, presents a paradox. Moreover, neither slices nor homogenates of the tissues mentioned inactivate the hormone upon incubation at physiological pH for 4 hours at 37.5°C. This system may be expected to provide much additional material for study of the biosynthesis, distribution, fate and mechanism of action of this vital hormone of normal and abnormal pregnancy.

(c) Appropriate studies have indicated that, unlike normal placenta, choriocarcinoma is not productive of detectable amounts of estrogen, progesterone, or corticoid.

(d) A hamster in which a choriocarcinoma tumor has become resorbed completely resists reinoculation with the same tumor strain. The serum of such a resistant hamster will inhibit the tumor from taking in a previously uninoculated hamster, thus demonstrating the passive transfer of immunity to a heterologously transplanted tumor. That this is not the effect of non-specific anti-human serum is shown by the failure of potent anti-human-globulin rabbit serum to transmit such resistance to tumor inoculation. However, much needs to be done to clarify the true nature of this interesting effect.

Major Findings (con't.)

In collaboration with Dr. Bosisevich of NIAID we have failed to demonstrate any significant human antigenicity of choriocarcinoma tissue when administered in Freund's adjuvant to the rabbit or by tumor implantation in the hamster. These tests for anti-human substance were carried out by complement fixation, Ouchterlony plate and by Bentonite flocculation. It would seem that this primitive embryonal tissue lacks the capacity to induce anti-human antibodies in the rabbit and hamster and yet can impart tumor resistance in the hamster. The data are suggestive of a tumor-specifit resistance factor not related to orthodox anti-bodies. Further studies are in progress to identify this factor.

(e) The availability of one of these tumor strains on a weekly turnover basis has permitted the development of a chemotherapeutic screening procedure against this strain. A wide variety of hormonal agents of estrogenic, androgenic, progestational and corticoid type have been found not to affect the normal growth rate of the tumor. This strain was derived from a methotrexate-resistant patient but the transplanted tumor exhibits a moderate sensitivity to methotrexate in sub-lethal doses.

The periwinkle plant, Vinca rosea, has been found to contain several ingredients of alkaloid type which are highly inhibitory to the tumor, the most potent of these being now identified as Vincaleukoblastine and Leurosine. Related alkaloids derived from this plant such as Lochnerine, perivine, reserpine, catharanthine, and vindoline are totally inactive at toxic dose levels. These observations have been extended to the therapy of human choriocarcinoma (cf. 803).

In addition an extended series of unrelated alkaloids have been tested, all with negative effects.

(f) A tissue culture laboratory has been developed and numerous methodological problems worked out. Choriocarcinoma from the hamster has been successfully planted in roller tubes and T-flasks. Hormonally active supernatant fluid has been repeatedly recovered. Quantitative studies have been undertaken to determine rates of hormone-production in vitro. These in vitro systems will provide excellent material for analyzing the nutritional and environmental requirements to hormone formation and the relation of hormone formation to cellular proliferation and differentiation.

2. Chorionic gonadotropin has been identified only in the horse, monkey and chimpanzee. In the Macacus rhesus monkey we have found detectable levels in the serum and urine during pregnancy only between day 20 and day 35 following timed mating. The hormone then abruptly becomes undetectable even in extensively concentrated plasma or urine. It is also absent from peripheral tissues such

Major Findings (con't.)

as liver, kidney and spleen throughout pregnancy. Although it is readily detectable in the fetal chorion as long as the peripheral plasma remains positive, it is no longer detectable in this tissue which is usually regarded as its site of formation after day 35 of pregnancy. It is possible that it is present in a form which becomes unavailable to the test rat or mouse. It is also possible that the levels may fall below that detectable by our current assay methods. Further attempts at concentration of large pools of plasma and chorionic tissue late in pregnancy will be undertaken, since the period of detectability in the monkey corresponds to the peak of quantitative output in human pregnancy. In the human, however, easily detectable levels persist throughout pregnancy.

The presence of hormone from day 20 to day 35 of pregnancy in monkey plasma and chorion has provided a tool for the evaluation of survival capacity of ectopically transplanted chorionic tissue in this species. Since these matings are not brother to sister it was deemed desirable to attempt to depress the maternal "resistance" to ectopically placed chorionic tissue by prior immunization of and cortisone administration to the mother which was to receive her own chorionic villi. Various routes of dispersion of villi have been tried in a total of 12 such pre-treated pregnant monkeys, but all have been directed toward distributing the villi throughout the pulmonary bed via the right heart. In no instance has there been hormonal or histological evidence of a take. In fact, no villi have ever been recovered at subsequent autopsy after periods ranging from 30 to 150 days. Thus our attempts to experimentally reproduce hormone-producing ectopic villi with potential malignancy have met with complete failure -this far.

3. Further studies on the biological properties of human chorionic gonadotropin have been directed toward the development of more sensitive and less laborious methods for bioassay. One of the initial effects of the hormone is to produce an ovarian hyperemia which is grossly visible but not quantitatively assessable by the naked eye. Radioiodinated human serum albumen has been found to increase in the hyperemic ovary at a rate which is proportional to the amount of chorionic hormone administered. This provides increased sensitivity over the mouse assay and with proper automatization may provide a very practicable method for the numerous assays which our clinical and biological work requires. The initial data are encouraging and further efforts toward the development of this assay are being pursued.

4. Previous studies have indicated that the ovary of the rat and rabbit undergo a substantial degree of differentiation and growth prepubertally. This process is pituitary-dependent since it will progress normally in ovarian grafts in hypophysectomized male or

Major Findings (con't.)

female hosts. We have attempted to determine the role of the hypothalamus in this process. Extensive hypothalamic destruction in the rat is without effect upon pre-pubertal gonadal development. Further studies of possible neural factors involved in this process are in progress.

5. Further studies of the functional potential of pituitary grafts in hypophysectomized rats show that these ectopic glands produce ACTH, growth hormone, and gonadotropin without hypothalamic regulation.

6. The Dunning rat leukemia which has previously been carried in Fischer rats has been successfully adapted to the Sprague-Dawley rat and is now in the 70th transfer generation in this strain. It has developed increased "virulence" with this adaptation since it kills in 7 to 9 days in the Sprague-Dawley strain and in 14 to 17 days in the Fischer strain.

This tumor is sensitive to methotrexate and to hydrocortisone. Combination studies have clearly shown that half-maximally effective doses of each of these drugs are not at all additive. Hence it is clear that each of these agents produces its inhibitory effect by a distinct mechanism.

It has been shown microbiologically that biotin deficiency impairs the capacity of numerous microorganisms to synthesize folic acid. Attempts have been made to determine whether biotin deficiency in the leukemic rat will reduce the requirement for methotrexate for maximal inhibition of tumor growth. No such relationship could be demonstrated nor did the partial biotin deficiency produced by egg-white diets impair the growth of the tumor significantly.

7. We have found that if an immature female rabbit is first treated with one-tenth of the effective dose of follicle-stimulating hormone for 10 days, then subsequent administration of even excessive doses of the same hormone proves totally ineffective. This we have called a desensitization phenomenon. Further studies have indicated that cortisone blocks this desensitization phenomenon whereas antihistaminics do not do so. This suggests a possible immune mechanism and further experiments will help to elucidate this problem.

Proposed Course of Project: It is expected to continue and extend the foregoing studies along the lines already indicated.

Part B included.

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Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Goodman, A. D.: Effects of Pantothenate Deficiency and of Omega-Methylpantothenate on Rat Corticosterone Secretion. Federation Proc. 18: No. 1, March 1959.

2. Hertz, R.: Choriocarcinoma of Women Maintained in Serial Passage in Hamster and Rat. Proc. Soc. Exper. Biol. & Med. 102: 77-81, 1959.

3. Hertz, R.: Growth in the Hypophysectomized Rat Sustained by Pituitary Grafts. Endocrinology (in press).

Honors and Awards relating to this project:

Dr. Roy Hertz

Elected member of the Research Advisory Council of the American Cancer Society for a term of 5 years.

Member, Endocrinology Panel of the Cancer Chemotherapy National Service Center.

Participated in conference on The Biological Activities of Steroids in Relation to Cancer, CCNSC, Vergennes, Vt., September 1959.

Guest speaker, Southwest Section of American Association for Cancer Research, Inc. (Noble Foundation), Ardmore, Oklahoma, October 16, 1959.

Chairman, Panel on Endocrine Aspects of Cancer Palliation, Annual Postgraduate Assembly of the Endocrine Society, San Francisco, California, November 1959.

Serial No. NOI-610
1. [illegible]
2. Endocrinology Serv[illegible]
3. Bethesda, Md.

FWS-VIR
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pharmacological and Physiological Studies on Alteration of Endocrine Function in Animals.

Principal Investigator: Dr. William W. Tullner

Other Investigators: Dr. Roy Hertz

Man Years (calendar year 1959):
Total: 3 1/4
Professional: 1
Other: 2 1/4

Project Description:

Objectives: 1. To evaluate selected synthetic compounds for the ability to alter endocrine secretions in order to explore the potential usefulness of such compounds in the control of endocrine tumors. To develop and improve biological and biochemical methods of steroid determination.

2. To investigate the mechanism of action of compounds which inhibit steroidogenesis employing quantitative and qualitative methods for determination of alterations in the intermediate and final products of steroid biosynthesis.

3. To investigate the interrelationship between the anterior pituitary, adrenal cortex and the reproductive system particularly with respect to the secretion of androgens and estrogens by the adrenal cortex.

4. To induce adrenal cortical tumors in laboratory animals for the purpose of studying steroid production of such tumors and means of inhibiting tumor growth.

Methods Employed: Normal healthy animals were used in these experiments. Stock diets were used except for special post-operative reasons or experiments related to nutritional effects on hormone secretion.

Both standard surgical techniques and those which have been developed for these studies have been employed. These include adrenal vein cannulation, hypophysectomy, adrenalectomy, ovariectomy and endocrine organ transplants.

Standard chemical methods were used for the quantitative assay of steroid hormones.

Major Findings: 1. The following findings are based on pharmacological studies with o,p' DDD (2,2-bis(2-chlorophenyl-4-chlorophenyl-1,1-dichloroethane).

A. In our previous experimental studies, the problem of incomplete absorption was frequently associated with the oral administration of fat-soluble compounds such as o,p' DDD. This has led to development of a suitable vehicle for the intravenous administration of such compounds (Publication No. 3).

Using this intravenous method for the investigation of the effects of o,p' DDD in several series of dogs, it was found that after 48 hours of treatment, the rate of secretion of adrenal cortical 17-hydroxy steroids decreased markedly. Preliminary observations indicate that there is also a profound reduction in secretion rates of corticosterone and aldosterone so that with respect to the major end products of the dog adrenocortical biosynthetic pathway, the effects of this drug resemble those following the disappearance of adrenocorticotropin after hypophysectomy. However, in no instance could this inhibitory effect on adrenocortical steroid output be reversed by adrenocorticotropic hormone indicating that the drug acts directly on the adrenal cortex. Intravenous studies with o,p' DDD are being conducted currently to determine: effects of time - dose alterations as related to inhibition of adrenocortical function; methods for increasing concentration of the compound per unit volume of vehicle; and its distribution in the body tissues of the dog.

The development of an intravenous emulsion containing o,p'DDD makes possible acute experiments which may elucidate the mechanism by which this drug exerts its action. Several adrenocortical metabolites are to be tested for their ability to block the inhibitory action or reverse the pharmacological effects. We propose to investigate the effects of mevalonic acid, squalene, cholesterol and pantothenic acid as related to the adrenocortical inhibitory action of o,p' DDD.

B. Since a method has been devised for the intravenous administration of fat-soluble compounds, it has been possible to begin the evaluation of other analogs of DDD particularly with respect to the action of these analogs on the adrenal cortex. In acute studies on the dog, it was found that difluorodinitrodiphenyldichloroethane inhibits 17-hydroxycorticosteroid secretion. Further comparisons with o,p' DDD are underway and other halogenated analogs are to be tested in an effort to find compounds of greater potency.

C. Previous investigations of the acute pharmacologic action of orally-administered o,p' DDD in the dog have been extended (Publication No. 1). Within 24 hours after a single oral dose of the drug, there was a complete suppression of 17-hydroxycorticosteroid output although there were no apparent histological changes in the

Major Findings (con't.)

adrenal cortical tissues. Exogenous adrenocorticotrophic hormone did not reverse this acute inhibition of adrenocortical secretion.

D. A series of dogs received chronic oral treatment with o,p' DDD for periods up to 90 days. After 90 days, the cell architecture of the adrenal cortex was completely destroyed. In animals that died in apparent adrenal insufficiency as long as 3 months after cessation of treatment, there was no evidence of adrenocortical regeneration such as we had previously observed at intervals following 3 weeks treatment at a comparable daily dose level.

E. Chronic, oral administration of o,p' DDD to the rhesus monkey (Macacus rhesus) had no observable effect on adrenocortical function. Adrenal venous blood levels of hydrocortisone of treated animals were within the normal range. There was no evidence of histological alteration of the adrenal cortex.

Although Nelson and Woodard had reported that treatment with technical DDD produced no histological change in the rat adrenal cortex, early functional effects, without concomitant histological change as observed in the dog, indicated that the problem warranted reinvestigation especially with the potent isomer o,p' DDD. Following chronic oral treatment with o,p' DDD, the adrenal secretion of corticosterone of the treated animals was not significantly different than in untreated animals.

The above findings in the monkey and rat further emphasize the marked species specificity of this compound.

2. In a continued investigation of the endocrine effects of diaminodiphenyl compounds, studies were limited to diaminodiphenylmethane (Methylenedianiline) (Publication No. 2). In the castrate female rat, chronic treatment with this compound produced adrenal hypertrophy, uterine enlargement and was goitrogenic. Histologically the adrenal cortex showed marked lipid accumulation. In the dog, acute intravenous administration of diaminodiphenylmethane produced a 7 to 8-fold decrease in 17-hydroxycorticoid output 5 minutes after administration. This decrease was accompanied by a concomitant transient increase in blood pressure. In the immature female rabbit, this compound elicited a progestational proliferation of the endometrium which was shown to be adrenal-dependent. In this respect, the compound is three times as active as emphenone. Adrenalectomy as well as either cortisone or corticosterone-treatment blocked the progestational effect of diaminodiphenylmethane.

Major Findings (con't.)

However, there was a lack of progestational response to the compound when the rabbits were not primed with estrogen. With the exception of the pressor qualities and a lack of anesthetic action, the effects noted above are similar to those of amphenone (3, 3-bis (p-aminophenyl)-2-butanone). These findings demonstrate that the methyl ketone structure of amphenone is not essential for the endocrine responses observed. Because of toxicity, no further studies are planned with this compound although the possibility of obtaining less toxic structurally-related compounds is not being overlooked inasmuch as the symmetrical diphenyl structure is common to several potent inhibitors of adrenocortical enzyme systems in laboratory animals and man.

3. Studies on the interrelationships of the anterior pituitary adrenal cortex and reproductive system have been continued.

A. The problem of sex hormone secretion by normal adrenocortical tissue and the relationship of adrenocortical secretion to the state of the reproductive system requires clarification. Preliminary experiments, in which rat adrenal glands were transplanted under the kidney capsule of castrate males contiguous to homologous transplants of seminal vesicles from other castrate males, indicate a degree of androgenic stimulation of the seminal vesicle secretory epithelium. It is proposed to determine the role of the anterior pituitary trophic hormones in this system, beginning with injection of whole rat pituitary extracts followed by combinations of adrenocorticotropin and the gonadotropins.

B. Although the uterine endometrium requires pretreatment with estrogen to obtain the optimum response to progesterone, it has been observed (Hisaw et al.) that high doses of progesterone alone can induce progestational proliferation of the rabbit endometrium. In order to determine whether adrenal estrogen secretion might be sufficient to induce the response to progesterone alone, adrenalectomized rabbits were treated with progesterone. Progestational proliferation of the endometrium occurred in the absence of the adrenals indicating that estrogenic hormone secretion by the adrenal is not a factor in the direct action of progesterone.

Chemical characterization of the adrenal compounds responsible for the progestational actions of amphenone and diaminodiphenylmethane is also a part of the proposed course of these studies.

4. Development of a direct method for the assay of adrenocorticotropic hormone (ACTH) was undertaken. Using the acutely hypophysectomized rat, it was found that the adrenal secretion of corticosterone was a sensitive indicator of injected ACTH at low dose levels. A similar method was described at the meetings of the Federation of American Societies for Experimental Biology (Lipscomb and Nelson). These studies have not been pursued further.

Major Findings (con't.)

5. Because the current experiments on the induction of adrenal tumors in the dog are of a long-term nature there is no evidence at present as to the success of experiments initiated within the past year. Postnatal ovariectomy of several litters of puppies has provided a series of animals which will be examined for adrenal pathology in the coming year. Likewise, a series of dogs received intensive chronic doses of o,p' DDD which were adequate for destruction of adrenocortical tissue. Treatment was concluded and after several months, biopsies will be taken to determine whether the rapid regeneration of adrenocortical tissue following drug treatment has resulted in cellular alterations.

Significance to Bio-medical Research and the Program of the Institute. Basic understanding of the functional interdependence of the adrenal cortex and the reproductive system and their respective steroid biosyathetic patterns add not only to our knowledge of normal function but also provide information needed for the pharmacologic control of abnormal growth and secretion. Compounds such as those reported here, which are highly selective in altering the function of endocrine tissues, offer a possible means for control of endocrine cancer.

Proposed Course of Project. It is expected that the above studies will be elaborated and continued as already indicated under Major Findings.

Part B included.

Serial No. NCI-810
Endocrinology Branch

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Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Vilar, O. and William W. Tullner. Effects of o,p' DDD on Histology and 17-Hydroxycorticoid Output of the Dog Adrenal Cortex. Endocrinology 65: 80, 1959.

2. Tullner, William W. Endocrine Effects of Methylenedianiline in the Rat, Rabbit, and Dog. Endocrinology (in press).

3. Tullner, William W. and Roy Hertz. Effect of Intravenous o,p' DDD on Plasma 17-Hydroxycorticoid Secretion in the Dog. Endocrinology (in press).

Honors and Awards relating to this project:

Elected Member of American Association for Cancer Research.

Results obtained from this research project were presented to the following professional groups:

1. The Endocrine Society, Atlantic City, N. J. June 1959.

2. The XXI International Congress of Physiological Sciences, Buenos Aires, Argentina August 1959.

Serial No. NCI-613
1. Endocrinology Branch
2. Endocrinology Service
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Preparation of Crude Natural Extracts of Hormonal Activity from Urine and Serum and their Purification and Physical-Chemical Characterizations.

Principal Investigators: Dr. Ralph A. Reisfeld, Dr. Roy Hertz and Dr. Charles F. Nadler

Cooperating Units: Laboratory of Biochemistry, NCI (Dr. E. A. Peterson)

Man Years (calendar year 1959):
Total: 3
Professional: 2
Other: 1

Project Description:

Objectives: To obtain crude preparations of chorionic gonadotropic hormone from the urine of pregnant women and of women with trophoblastic tumors. Purification and concentration of these hormones followed by characterization of their physical-chemical properties. Comparison of these properties of the gonadotropic hormones to determine whether or not there is a chemical difference between hormones with similar biological properties but obtained from normal and cancer sources, respectively. Furthermore, it would be of interest to determine as many physical-chemical characteristics of this hormone as possible since this could ultimately lead to a chemical assay method which has been so far unavailable.

Methods Employed: 1. Tumor tissue from patients with choriocarcinoma was extracted and fractionated according to the techniques of Sober and Peterson.

2. Cellular fractionation of freshly prepared choriocarcinoma tissue recovered as heterologous transplants from hamsters was carried out by the techniques of Hogeboom and Schneider.

3. Urinary concentrates were prepared following appropriate adsorption and elution procedures as prescribed by Drs. Sober and Peterson.

4. Bioassay of these fractions and concentrates were carried out by the mouse uterine weight method.

Major Findings: 1. The fractions from tumor tissue have been chemically and biologically characterized (Publication No. 1).

2. Preliminary data indicate that most of the hormonal activity of the tumor cell is in the small granular fraction with little in the supernatant fraction. No activity is detectable in the remaining cellular fractions.

3. Further chemical characterization of the chorionic hormone as recovered from the urine of tumor patients suggests some chemical differences from that obtained from normal pregnancy urine (Concentration of Gonadotropic Hormone from the Urine of Patients with Trophoblastic Tumors, R. Reisfeld and E. Hertz, abstract presented at meetings of the American Chemical Society, September 1959).

Significance to the Program of the Institute: The availability of non-toxic concentrates of naturally occurring hormonally active substances or of inhibitory anti-sera capable of neutralizing such endogenous biological effects in the cancer patient would permit therapeutic investigations along lines now neglected as a result of the emphasis on synthetic compounds.

Proposed Course of Project: Extension of these studies along the lines outlined above are projected and will be executed as appropriate personnel becomes available.

Part B included.

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Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Nadler, Charles F. and Reisfeld, Ralph A.: Gonadotropic Hormone Distribution in Protein Extracts of Trophoblastic Tumors in Man. J. Lab. & Clin. Med. (in press).

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Preparation and Evaluation of Biotin Antagonists in the Study of Biotin Metabolism as an Approach to Cancer.

Principal Investigator: Mr. Frederick G. Dhyse

Other Investigators: Dr. Roy Herts

Man Years (calendar year 1959):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives: The principal objectives of this project are to develop and prepare biotin antagonists that are particularly effective in producing deficiencies of this vitamin in laboratory animals as well as in microbial systems. A possible route of application of such deficiencies to cancer studies is described under "Significance to the Program of the Institute." Furthermore, appropriate antagonists are to be used to "block" certain biochemical reactions involved in the study of Biotin Metabolism in bacteria.

Methods Employed: Biotin antagonists are prepared by organic chemical synthesis or isolated from natural sources, and evaluated by microbiological assay and biological testing in rats and mice.

Major Findings: Further testing of Biotin Sulfone and γ(3,4-Ureylenecyclohexyl) butyric acid on rat leukemia did not results in significant inhibition of such leukemia.

Excess biotin, (above that required for maximum growth), added to cultures of L. arabinosus was found to give rise to a five-fold increase in secretion of folic acid by this organism into the surrounding medium. The folic acid was measured after 3 days incubation of L. arabinosus and at constant levels of maximum growth. It was expressly noted that excess biotin did not increase the growth response of the folic acid assay organism (L. casei). This effect seems to be specific for biotin-folic insofar as tested since an excess of pantothenic acid or riboflavin did not cause a significant increase in the secretion of folic acid by L. arabinosus.

Major Findings (con't.)

This folic increase was found to take place only in the fraction elaborated into the medium and not in the cells, where the folic acid content was found to be constant. Furthermore, this folic acid secretion was found only when the excess biotin was added at the start of incubation or within 24 hours thereafter. Biotin added after L. arabinosus had attained maximum growth did not result in any increased folic secretion.

These findings are of particular interest because of a previous report in the literature suggesting that biotin may be required for the tissue synthesis of folic acid in the rat.

A deuterium derivative of γ(3,4-Ureylenecyclohexyl) butyric acid, (UCB) prepared by substituting deuterium for hydrogen gas in the catalytic hydrogenation process by which UCB is usually prepared, did not show a significant increase of anti-biotin activity over that of the regular UCB.

Significance to the Program of the Institute: Evidence from the literature indicates biotin may be required for the tissue synthesis of folic acid, and findings presented in this report show a positive effect of biotin on the synthesis of folic acid in microorganism. In view of the relative success of folic antagonists in the treatment of cancer, and because of the drug resistance later developed to such treatment, an appropriate inhibitor of biotin's role in folic acid synthesis might make present treatment with anti-folics more effective.

Proposed Course of Project: 1. Further study of the relationship of biotin to the biosynthesis of folic acid in microorganisms. Development of antagonists to biotin and to intermediates in this particular biotin-folic function. Extension of such studies to laboratory animals for investigations of the possible tissue synthesis of folic acid.

2. Evaluation of biotin antagonists, and also antimetabolites of certain biotin vitamers in microbiological systems where drug resistance has been developed to anti-folics. Extension of such studies to rats and hamsters with tumors resistant to methotrexate.

3. Separation and isolation of the biotin vitamers described in a publication of previous findings in connection with this project.

Serial No. NCI-4-709 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.:

Project Title: The Chemotherapy of Acute Leukemia

Principal Investigators: Emil J Freireich and Emil Frei III

Other Investigators: L. Schroeder

Cooperating Units: Clinical Pharmacology and Experimental Therapeutics Service
Jefferson Medical College
Walter Reed Army Medical Institute
Jackson Memorial Hospital
Medical College of Virginia
Roswell Park Memorial Hospital
Emory University
Bowman-Gray School of Medicine
Mount Sinai Hospital, New York
Philadelphia Children's Hospital
Mayo Clinic
Dartmouth University Hospital

Man Years (calendar year 1959) Patient Days (calendar year 1959)
Total: 7
Professional: 5
Other: 2

Project Description:

Objectives: 1. To study quantitatively the optimum usefulness of drugs that are known to have activity against acute leukemia.
2. To discover and test in humans new chemical compounds which can alter the course of acute leukemia.
3. To study the effects of these compounds on the host.
4. To test the usefulness of animals and other screening techniques by the collection of quantitative clinical data in man.

Methods Employed:

1. Case Material: All patients referred to the Clinical Center with the diagnosis of acute leukemia are admitted to the study. In addition there are 12 hospital units other than the NCI participating in most of the below mentioned therapeutic trials.
2. Criteria: Objective criteria for the evaluation of disease activity have been developed and applied. Their applicability and meaningfulness is under continuing scrutiny.

Part B included YES

3. Pharmacology: In cooperation with the Laboratory of Clinical Pharmacology, pharmacologic disciplines are applied to the drugs tested. These studies are divided into three major categories:

a) Toxicity studies of compound of potential antitumor activity given to humans for the first time.
b) Absorption, distribution and excretion studies of known and potential antitumor compounds.
c) Analysis of the influence of varying dosage schedules and routes of administration of antitumor activity and toxicity.

4. Experimental Design: In cooperation with biostatisticians and the Clinical Pharmacology Section therapeutic trials were designed. These include:

A. Cooperative comparative therapeutic trial designed to obtain among other things, quantitative information of the relative effects of antimetabolites of proved antitumor activity in man.

B. Sequential Analysis - New drugs are tried in patients with acute leukemia who have already received conventional anti-leukemic therapy. If the pilot studies suggest possible human anti-leukemic activity further studies are designed. Initially these have been performed by administration of the drug to be tested to all patients qualifying.

Major Findings:

1. Protocol II - This is the second definitive comparative study undertaken by the group, and in it comparison is made between three treatment programs utilizing drugs that are known to have anti leukemic activity. 1) Methotrexate (MTX) followed by 6-mercaptopurine (6-MP); 2) 6-MP followed by MTX; and 3) MTX and 6-MP given in combination. To date 328 patients have been entered on this study for the entire cooperative group, 92 of these patients being studied at NCI. This study has just been completed, and although final analysis has not been completed. Preliminary evaluation of the data on 215 patients has revealed the following: 1. The highest remission rate occurs with combination therapy although this is not higher than the rate with 6-MP plus that with MTX. 2. The remission rate with either 6-MP or MTX is not different when given as initial therapy or as second phase therapy after treatment with the other agent. 3. 6-MP alone gives a higher remission rate than MTX alone whether given as initial therapy, or after a previous antimetabolite. 4. The overall remission rate for all programs is highest in children (50%) intermediate in the intermediate age group (30%) and low in the adults (15%). 5. MTX was essentially inactive in adults (<5%). 6. Toxicity requiring cessation of therapy occurred between 9 and 25 days with an average of 14 days and was comparable for all treatment programs. 7. Qualitatively MTX gave more oral ulceration and 6-MP more GI symptoms

2. Protocol III. - This is the third definitive comparative study undertaken by the group. The objective of this study is to develop a method for the evaluation of new agents in patients with acute leukemia, early in the course of their illness, before the use of 6-MP or MTX. The proposed method would measure the duration of a remission induced by adrenal steroids, to determine whether the antileukemic action of a drug can be detected by its ability to prolong such a remission. For this study an agent of proven activity (6-MP) was selected for study. All patients in remission from steroid therapy receive either 6-MP or placebo maintenance therapy and the duration of remission is measured. To date 59 patients have entered the study (11 at NCI). The data have not been analyzed.

3. Dichloroamethopterin - Following initial toxicity studies, a controlled comparative study of dichloroamethopterin and amethopterin was undertaken in patients less than 20 years of age who had received no prior antifolic therapy. To date, 9 patients have entered this study, (3 at NCI). These data have not been analyzed.

4. Cytoxan - This compound has been studied by the group with two major objectives: 1. to determine whether the compound is active against human leukemia, and 2. to determine whether dosage schedule effects the activity of this compound. In the L1210 mouse leukemia and other mouse and rat transplanted tumors cytoxan is highly active and activity is changed by alteration of dose schedule. To date 96 patients have entered the study (30 at NCI) 30% of patients have shown objective improvement, but in only 13% was this sufficient to qualify for partial remission. Dosage schedule (daily or weekly) did not show significant differences although somewhat more improvement was noted with the weekly schedule. Toxicity was not significantly different for the two schedules.

5. Methyl-glyoxal-bis-guanylhydrazone. This compound has been studied sequentially in patients with acute leukemia. 36 patients have entered the study (13 at NCI). To date, no favorable responses have been noted.

6. Narcotine - This compound is highly cytotoxic in tissue culture but inactive against animal tumors. 9 patients (5 at NCI) have received this compound. No beneficial effects have been noted.

7. 6-azauridine - This compound is the riboside of 6-azauracil. It has been studied in 4 patients (2 at NCI). It has been able to produce a fall in leukemic leukocytes in the peripheral blood, but this effect has been transient and reversible despite continued therapy. No neurotoxicity has been noted at doses of 20 times the maximal dose tolerated for 6 azauracil.

Proposed Course: 1. Protocol III may provide the chemotherapist with a powerful tool for the study of new chemotherapeutic agents in patients with acute leukemia. If so, it will be utilized as a design for studying new agents. 2. Studies of 6 Azauridine will proceed. 3. Studies of the effect of route of administration on the action of antifolic compounds such as dichloroamethopterin will be undertaken.

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Part D.: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Shnider, B. I., Fred, E. III, Touby, I. H., Garman, J., Freireich, E. J, Brindley, C. O., and Clemente, I. Observations on the human toxicity and pharmacologic activity of 6-Azauracil. Cancer Research, in press.

Honors and awards relating to this project: NONE

Serial No. NCI-4-715 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Mechanism and Management of the Hemorrhagic Disorder Associated with Acute Leukemia

Principal Investigator: Emil J Freireich, M.D.

Other Investigators: L. R. Schroeder, M.D. and Emil Frei III, M.D.

Cooperating Units: Pathological Anatomy, N.I.H., Dr. L. Thomas, Blood Bank, D.B.S., Dr. Kliman, Dr. P. Schmidt

Man Years (calendar year 1959) Patient Days (calendar year 1959)
Total: 2
Professional: 1
Other: 1

Project Description:
Objectives: a) To identify factors responsible for precipitating gross hemorrhage in thrombopenic acute leukemia patients.
b) To study the role of the platelet in blood coagulation, and hemostasis.
c) To improve the therapy of thrombopenic hemorrhage.

Methods Employed:
a) In vitro and in vivo blood coagulation studies.
b) Column chromatography using cation exchange resins.
c) Analysis of clinical, laboratory and pathological data on patients with hemorrhage.

Major Findings:

1. Purification of Thrombin - Purified thrombin is poorly soluble. At low ionic strengths and at ph values below 6.5, the protein is irreversibly precipitated. This characteristic has made the isolation and concentration of sufficient protein for further chemical study very difficult. Progress has been made. By appropriate selection of ph and ionic strength values, batch preparation procedures have been improved. These may lead to isolation of sufficient material from both bovine and human sources to allow for further chemical study.

2. Antithrombin thrombin interaction - Human thrombin is less stable than Bovine thrombin in both human and bovine plasma. This is true when thrombin activity is estimated by either clotting human, or bovine fibrinogen or by its esterase activity on Tosyl arginine methyl ester. For both enzymes the rate of loss of activity

is a function of concentration of enzyme added. When thrombin is added to plasma further conversion of prothrombin to thrombin is decreased in proportion to the quantity of thrombin added.

3. Review of clinical and laboratory data on a group of patients with acute leukemia has been undertaken to define the relationship of clinical hemorrhage to platelet levels, fever, infection, organ size, etc. Preliminary analysis indicates that platelet level correlates best with clinical hemorrhage. Hemorrhage rarely occurs when platelet levels are above 20,000 per cu.mm. and occurs more regularly at levels below 5,000 per cu.mm.

Proposed Course of Study:

1. Studies of purified thrombin and the thrombin antithrombin interaction will be continued. The biological activity of the products of this interaction will be studied.

2. In collaboration with the Clinical Center Blood Bank, D.B.S., we have initiated studies of fresh platelet transfusions in patients with acute leukemia. The platelets are obtained by plateletpheresis from selected donors. This technique may allow for greatly increased availability of fresh platelets which has hitherto limited our studies in this area. If this source is an effective one we plan to study the effect of prophylactic platelet transfusions on hemorrhage and survival of patients with acute leukemia.

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Part D. Honors, Awards, and Publications

1. Fritz, R. D., Forkner, C. E., Jr., Freireich, E. J., Frei, E. III, Thomas, L. The association of fatal intracranial hemorrhage and "blastic crisis" in patients with acute leukemia. New Eng. J. of Med. 261:59-64, 1959.

2. Freireich, E. J., Thomas, L. B., Frei, E. III, Fritz, R. D., and Forkner, C. E., Jr. A distinctive type of intracerebral hemorrhage associated with "blastic crisis" in patients with leukemia. Cancer. In press.

Serial No. NCI-4-719 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Study of the Clinical Manifestation of Acute Leukemia.

Principal Investigators: Emil J Freireich, M.D. and Emil Frei III, M.D.

Other Investigators: L. R. Schroeder, M.D., Richard Shaw, M.D., Edward Moore, M.D., Dane Boggs, M.D. and Arthur Teplitsky, M.D.

Cooperating Units: None

Man Year (Calendar Year 1959): Patient Days (Calendar Year 1959):
Total: 2
Professional: 2
Other: 0

Project Description:

Objectives: To delineate etiologic and pathogenetic factors of the various manifestations of acute leukemia.

Methods Employed: The comprehensive application and correlation of clinical, hematological, radiologic, chemical, microbiological, and histologic methods to the above manifestation in patients with acute leukemia.

Major findings:

1. Meningeal and Central Nervous System Involvement. A retrospective clinical-pathological study of meningeal leukemia in 150 patients with acute leukemia has been completed. The clinical syndrome of meningeal leukemia which results primarily from increased intracranial pressure was recognized in 25 (17%) of the 150 patients studied. It occurs primarily in children with acute lymphocytic leukemia. Eight of the 25 patients developed the first evidence of meningeal leukemia while in remission. Review of the literature indicates a sharp increase in the frequency of this syndrome since the introduction of chemotherapy. These data suggest that the increasing importance of this clinical syndrome results from the use of chemotherapeutic agents effective against the systemic disease but relatively ineffective against the meningeal involvement. Study of the central nervous system in these patients revealed that the syndrome of meningeal leukemia was associated with a high frequency of internal communicating hydrocephalus and with leukemic infiltration of the arachnoid.

Part B included NO

Leukemic involvement of the dura, choroid plexus or arachnoidal granulations was not related to the occurrence of meningeal leukemia.

2. Natural History of Disease. A review of the clinical course of 178 patients has been undertaken. Acute myelogenous leukemia was found in all age groups while acute lymphocytic leukemia was found primarily in children. (Under 20 years of age). Only 20% of the patients with acute myelogenous leukemia showed any improvement with chemotherapy. The response rate for patients with lymphocytic leukemia, however, was much better. More than 90% of the children with acute lymphocytic leukemia responded to chemotherapy. In contrast only 40% of the adults (greater than 20 years of age) with lymphocytic leukemia responded. The frequency of response to chemotherapy correlated with survival of the patient. Median survival for children with lymphocytic leukemia being 11 mos., and for adults with lymphocytic leukemia, 6 mos. Survival also related to the extent of remission. Median survival for children having a complete remission being 13 mos., and median survival for children showing no improvement being 2 months.

Significance to Cancer Research: The continuing scrutiny and definition of clinical manifestations of acute leukemia is of significance to cancer research from many points of view. This is one of the major areas wherein leads to pathogenetic mechanisms may be uncovered. In addition, precise observations are essential to defining the natural history of the disease and this latter is of critical importance to drug evaluation, the major effort of our section.

Proposed Course of this Project: 1. Studies of meningeal leukemia will be extended by study of blood brain barrier, ability of chemotherapeutic agents and other materials to enter the C.N.S. In addition a prospective study to assess the frequency and management of this syndrome will be undertaken.

2. Analysis of data concerning natural history will be completed, and further data accumulated.

3. Nucleic acid metabolism. The determination in the urine of purine and pyrimidine bases as well as nucleosides and nucleotides has recently become possible after 3 years of extensive methodological investigations carried out by the Laboratory of Physiology (See their Project Report). Studies in man are now underway comparing normals with patients with untreated acute leukemia.

4. Bone lesions. A comprehensive retrospective study of the clinical and x-ray manifestations of bone lesions in acute leukemia was conducted in 85 patients. In 20 of these patients the entire femur was removed at autopsy and the pathologic findings correlated with the clinical and radiological findings. Five distinct types of lesions have been encountered and their pathogenesis has been, in part, elucidated.

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Part B. Honors, Awards, and Publications

1. Moore, E. W., Thomas, L. B., Shaw, R. K., and Freireich, E. J. The central nervous system in acute leukemia: A postmortem study with particular reference to hemorrhages, leukemic infiltrations, and the syndrome of meningeal leukemia. A.M.A. Arch. of Int. Med. In press.

2. Wilbish, T. F., Besse, B. E., Lusted, L. B., Daves, M. L., Thomas, L. B., and Forkner, C. A. Acute leukemia. A. M. A. Arch. of Int. Med. 104:741-747, 1959.

Serial No. NCI-4-734 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.:

Project Title: Assay of Erythropoietin in bone marrow suspensions

Principal Investigators: L. R. Schroeder, M.D.
C. W. Gurney, M.D.

Cooperating Units: Argonne Cancer Research Hospital and Department of Medicine, The University of Chicago Clinics, Chicago, Illinois

Man Years (Calendar Year 1959) Patient Days (Calendar Year 1959)
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: To perfect a simple in vitro assay method for erythropoietin.

Methods: Suspensions of living hematopoietic cells from the bone marrow or spleen of suitable donor animals were incubated with radioactive iron in the presence of test and control plasmas. Cell-bound radioactive iron was measured in a scintillation counter.

Patient Material: Plasma samples from patients with a variety of hematologic disorders were assayed.

Major Findings: Human erythropoietin could be assayed by this technique and the demonstrated activity was independent of the iron content of the plasma. Modification of the originally-described assay and rigid control of iron concentration in the test system was necessary to achieve this result, but the additional steps involved did not vitiate the utility of the assay. The sensitivity of the assay appeared to be greatly increased by the use of hematopoietic cells from animals whose endogenous erythropoiesis was suppressed.

Significance to the Program of the Institute:

The humoral regulation of bone marrow activity is a basic physiologic mechanism as yet poorly understood. Knowledge of the factors influencing bone marrow response to stress and disease is fundamental to an understanding of the response to chemotherapeutic agents.

Part B Not Included

When the assay method is ready for application its economy of materials and simplicity should permit detailed investigations of erythropoiesis in health and disease that have hitherto not been feasible.

Proposed Course of Project:

As the major findings are the results of experiments considered preliminary and exploratory, they require confirmation. Upon confirmation, a detailed study of the kinetics of the assay is proposed, followed by a comparison of this technique with existing assay methods to define its accuracy and applicability.

Serial No. NCI-4-735 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Effect of Therapy on the Synthesis of Desoxyribonucleic Acid by Leukemic Cells

Principal Investigators: Dr. George Brecher
Dr. L. R. Schroeder

Other Investigators: Dr. Eugene Cronkite

Cooperating Units: Hematology Service, Dept. of Clinical Pathology, Clinical Center

Man Years (Calendar Year 1959)
Total: 1
Professional: 3/4
Other: 1/4

Project Description:

Objectives: To study the rate of DNA synthesis of leukemic cells of the peripheral blood and bone marrow in an in vitro system

Methods: Incubation of leukemic cells with tritium-labelled thymidine and identification of labelled cells by radioautography was used as an indicator of DNA synthesis.

Patient Material: Multiple peripheral blood and bone marrow specimens were obtained from patients with acute leukemia being cared for by the Chemotherapy Service of NCI.

Major Findings: The synthesis of DNA by leukemic cells continues in vitro for at least four hours with little or no diminution in rate. No new cells enter the synthetic phase of the generative cycle during in vitro incubation, and none divide.

The addition of large amounts of labelled thymidine to the system has no apparent deleterious effect and permits the preparation of satisfactory autographs in less than a week.

There is no correlation apparent between DNA synthetic activity and the usual criteria of cellular immaturity.

Part B NOT INCLUDED

There is a wide range in rates of DNA synthesis by leukemic cells. In none of the patients' cells examined did the rate approach what might have been expected, and in no case of leukemia did the rate in the cells of the peripheral blood exceed that of cells from patients with infectious mononucleosis.

Significance to Cancer Research:

It is now feasible to explore the effect, if any, of antileukemic therapy on DNA synthesis as measured by this system.

Anticipating success or failure in the treatment of acute leukemia with a specific agent is now possible only in a statistical fashion, meaningless for the individual patient.

If the effect of therapy on the rate of DNA synthesis by leukemia cells is found to predict eventual responsiveness of the individual's disease, it could result in a tremendous saving in time and patient morbidity.

Proposed course of the project:

Now that some knowledge of the in vitro kinetics has been gained, the procedure will be used to measure the effect of anti-leukemic therapy on cellular DNA synthesis and attempt to correlate this with remission of the disease.

Serial No. NCI-4 736 (c)
1. General Medicine Branch
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Demonstration of a Hormone Governing the Production of Platelets

Principal Investigators: L.R. Schroeder, M.D.
Glenn R. Lee, M.D.
Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959) Patient days (Calendar year 1959)
Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:
Objectives: To demonstrate the existence in man and animals of a hormonal substance which governs the production of platelets by the bone marrow.
Methods: Thrombopenia was produced in experimental animals by injections of saponin, by the passage of blood over columns of Dowex-50 resin beads, by prolonged thrombophoresis through an indwelling vascular catheter, and by the administration of specific platelet antibodies.

Major Findings:

Severe thrombopenia could be regularly produced by thrombopheresis or the administration of platelet antiserum with negligible side effects.

Plasma harvested at intervals from thrombopenic animals, pooled, and administered in large amount to normal animals resulted in one case in marked megakaryocytic hyperplasia of the bone marrow of the recipient. Control plasmas, saline, and plasma from thrombopenic animals harvested at different time intervals did not have a similar effect.

Significance to the Institute:
Proof of the existence of a hormone governing platelet production will add substantially to an understanding of the homeostatic mechanisms that control bone marrow function in the normal. Derangement of these mechanisms may have a role in the thrombopenia that is a part of the acute leukemias and the thrombocytosis that accompanies chronic myelocytic leukemia.

Proposed course:
Confirmatory experiments are in progress. If they support the reported observations, two lines of investigation are proposed.

Part B included NO

(1) repetition and expansion of the initial type of experiment to establish the statistical validity of the hormone's existence and to define the time-course of its appearance and utilization, as well as the threshold and maximum stimuli for its production.

(2) investigations in smaller experimental animals to develop an economical assay that is not species specific. Various maneuvers will be employed to enhance the responsiveness of the recipient animals in attempting to increase sensitivity and specificity of the assay.

When the assay system is developed, a survey of human plasmas for activity will be made. The collection of such plasmas has been begun.

Serial No. NCI-5-712(C)
1. General Medicine Branch
2. Solid Tumor Service, Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

A. Comparative Trials of Chemotherapeutic Agents in Patients with Solid Tumors.

B. Initial Therapeutic and Pharmacological Studies of Chemotherapeutic Agents in Patients with Solid Tumors.

Principal Investigators: Clyde O. Brindley, M. D. and Emil Frei, III, M. D.

Other Investigators: Montague Lane, M. D., Harold Silberman, M. D., James Marsh, M. D., Richard Mechanic, M. D. and Harold Fallon, M. D.

Cooperating Units: 1) Clinical Pharmacology and Experimental Therapeutics Service. 2) The Eastern Solid Tumor Chemotherapy Group.

Man Years (Calendar year 1959) Patient Days (Calendar year 1959)
Total: 3 years
Professional: 1 1/2 (E.F. 1/2 year., C.O.B. 1 yr.)
Other: Technicians 1 1/2 years

Project Title:

A. Comparative Trials of Chemotherapeutic Agents in Patients with Solid Tumors.

Project Description:

Objectives: To take part in the Eastern Solid Tumor Chemotherapy Group in the application of the principles of the Comparative Clinical Trial in the evaluation of chemical agents in the treatment of human cancer. The present primary objectives of the group are the improvement in the methodology of the Comparative Clinical Trial in human cancer patients and the use of the Comparative Trial to establish the relative effectiveness of selected chemotherapeutic agents in man.

Part B included Yes [X] No [ ]

These data can be used to (1) evaluate the correlation between the activity of these agents in animal tumor screening systems and in patients with cancer and (2) to establish their relative usefulness in the current therapy of human cancer.

Methods Employed:

These are undergoing continuing modification with experience. The cancer chemotherapist, pharmacologist and biometrician operate as a unit in these studies.

1) Patient Selection. The effect of prior therapy and extent of disease on response to therapy is being increasingly defined as data from quantitative studies accumulates. For studies involving direct comparisons it is desirable to use sensitive tumors (Lymphoma). For studies of insensitive tumors recent historical controls may be satisfactory.

2) Randomization. This will become more effective as factors influencing response to therapy are defined. These factors can be quantitatively assayed by regression analysis.

3) Determination of Effect and Analysis of Data. Standard criteria for response to therapy have been developed. Objective changes in tumor size from the basis of these criteria though symptomatic alterations will be recorded and analyzed in parallel.

Case evaluation with emphasis on quantal as well as qualitative responses has been improved. Evaluated cases will be coded on IBM cards. This should allow for rapid and complex interim analyses of studies, a procedure which in the past has been cumbersome. In addition, a complete, up-to-date analysis of all studies will be made at each meeting. This will be facilitated by the use of Status Reports for all patients still on study.

Major Findings:

1) Thiotepa (TSPA) - nitrogen mustard (HN_2) study - The first portion of this study indicated that in Hodgkin's disease HN_2 produced a somewhat greater remission rate and the remissions produced were significantly longer. However, as there was some evidence that the dose of HN_2 in this study was closer to the maximally tolerated dose, it was decided to obtain dose-response information for both TSPA and HN_2. It became quickly apparent that the dose-response curve was steep, i.e. that smaller doses were relatively ineffective. In addition, it would appear that at maximally tolerated doses the therapeutic effect of HN_2 and TSPA are similar.

2) Myeloma Study. The first myeloma study involving the comparison of azaserine and a placebo has been completed. Azaserine is inactive. In the current study urethane and a placebo are being compared. It is anticipated that the natural history of myeloma and the place of urethane in its treatment (a highly controversial area) will be quantitatively defined.

With this background it is planned to examine drugs that are active against mouse myeloma (a disease which is morphologically and biochemically very similar to human myeloma).

3) X-irradiation and HN_2 - The place of these therapeutic modalities in the treatment of lung cancer is inadequately defined. The relative effect of these agents, alone and in combination, on tumor size and survival is being studied.

4) Lymphoma Study. Dichloromethotrexate, methotrexate (both at two dose levels) and HN_2 are being compared in lymphosarcoma, reticulum cell sarcoma and Hodgkin's disease. Preliminary analysis indicates that the folic acid antagonists have antitumor properties comparable to HN_2 against lymphosarcoma and reticulum cell sarcoma. As with HN_2 the dose response curve is steep.

Project Title: B. Initial Therapeutic and Pharmacological Studies of Chemotherapeutic Agents in Patients with Solid Tumors.

Objectives: 1) To obtain information on the optimum route and mode of administration, toxicity and spectrum of antitumor activity of new chemotherapeutic agents in cancer patients. 2) To explore new methods for the determination of growth rate of tumors in man.

Methods: For the first objective the procedures are as follows: First the writing a protocol for the initial trial of the drug using the data available from animal antitumor and toxicity trials as guides. Then dose routes and dose levels are explored while observations on patient reaction, organ function and tumor growth rate are observed. Drug or tumor metabolic products may be followed. When the best route of administration, the primary limiting toxicity and maximum tolerated dose of an agent have been determined, the drug is given in two or three dose levels to patients with a broad spectrum of tumor types while observations for antitumor activity are made.

For the second objective serum enzyme levels and tumor metabolic products are assayed and tumor masses are measured directly as patients receive therapy. A correlation between changes in serum enzyme levels or tumor metabolic product excretion and changes in tumor growth rate are sought. The tumor metabolite and enzyme studies involve the use of the spectrophotometer, radioisotopes and the usual biochemical techniques.

Patient Material: For the exploratory drug toxicity and antitumor effects only patients with cancer or leukemia for whom other more conventional therapy offers little prospect of palliation are acceptable. For the studies on tumor growth rates, patients with many types of cancer and on various therapeutic regimens are included

Major Findings: Studies which have been completed in the last year are as follows:

1) Toxicity and Antitumor Trial of Uracil Mustard.
2) Toxicity and Antitumor Trial of Cytoxan.
3) Toxicity and Antitumor Trial of Monochlormethotrexate.

1) The optimum oral dose of Uracil Mustard was found to be 0.2 mg. per kilogram once weekly. Intravenous use of this agent was not explored. It had antitumor activity in Hodgkin's disease, lymphosarcoma and the chronic leukemias. It caused severe hemopoietic depression of both leukocytes and thrombocytes and occasionally very marked gastrointestinal disturbances.

2) Cytoxan (Cyclophosphamide) was tolerated by most patients at oral doses of 4 mg. per kilogram daily for several weeks. Intravenously the maximum tolerated dose for several weeks was 20 mg. per kilogram once weekly. Antitumor activity was seen in lymphosarcoma and ovarian carcinoma. The drug depressed the leukocytes relatively more than the thrombocytes. Alopecia was troublesome.

3) Monochlormethotrexate caused pronounced folic acid antagonist toxicity and impairment of hepatic function at doses of 0.05 mg. per kilogram daily within one to two weeks.

In those patients with metastatic cancer in whom the levels of the serum enzymes lactic dehydrogenase or ribonuclease were elevated, these enzyme levels decreased significantly when tumor regression was brought about by effective therapy. Limitations of these tests as indicators of tumor growth rates are that in some terminal patients these serum levels also fell when therapy was not effective. Tumor growth was being curtailed by nutritional changes in these patients. Also, many patients with metastatic cancer have no elevations prior to treatment and subsequently no changes in these enzymes during therapy.

Significance to Cancer Research: A necessary preliminary to a clinical trial of a promising agent is a determination of its route of administration, primary side effects and probable optimum dose range.

Indirect methods of following tumor growth rates would facilitate the trial of new agents in patients in whom the neoplasms are not directly measurable; this is the case particularly for many tumors arising from the gastrointestinal tract.

Proposed Course of Project Studies now in progress:

1) Toxicity study on parenterally administered dichlormethotrexate and methotrexate.
2) Antitumor trials in patients with malignant carcinoid.
3) Serum enzyme studies in solid tumor patients with measurable tumor masses during therapy.
4) Cellular enzyme studies on patients receiving pyrimidine antagonists.

In 1) above the two drugs are being given parenterally for several weeks, in preparation for a comparative antitumor trial using the parenteral route for drug administration. In 2) the urinary excretion of 5-hydroxyindole acetic acid is being followed as an index of tumor activity while patients are given new antitumor agents. In 3) these studies are being extended to include other enzymes, glutathione reductase and ribonuclease in an effort to find one which would be sensitive in gastrointestinal and genitourinary carcinomas. In 4) above the cellular enzymes concerned with pyrimidine metabolism are being studied. The site of action of certain pyrimidine antagonists can then be sought.

As with the comparative clinical trials continuing efforts are being made to improve our methods of conducting these preliminary studies.

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Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

1. Lane, L, Petering, H. G., and Brindley, C. O. Synthesis, Pharmacology and Clinical Trial of the Riboflavine Analogue, Sodium-6,7-Dimethyl-9-(2'-Hemisuccinoxyethyl)-Isoalloxazine, U-6538. J. Nat. Cancer Inst. 22, No. 2 349-361, 1959.

2. Brindley, C. O., Markoff, E., and Schneiderman, M. A. Direct Observation of Lesion Size and Number as a Method of Following the Growth of Human Tumors. Cancer Vol. 12, 139-146, 1959.

3. Zubrod, C. G., Scheiderman, M., Frei III, E., Brindley, C. O. Gold, G. L., Shnider, B., Oviedo, P., Gorman, J., Jones, R. Jr., Jonsson, U., Colsky, J., Chalmers, T., Dederick, M., Holland, J., Selawry, O., Regelson, W., Lasagna, L., Owens, A. Appraisal of Methods for the Study of Chemotherapy of Cancer in Man: Comparative Therapeutic Trial of Nitrogen Mustard and Thiotepa. J. Chron. Dis. In press.

4. Gold, G. L., Hall, T. C., Shnider, B. I., Selawry, O., Colsky, J., [illegible] A. M., Jr, Dederick, M. M., Holland, J. F., Brindley, C. O., [illegible] Cancer Research 19 (9): 935-939, 1959

rial No.NCI 5-737 (c)
1. General Medici
2. Chemotherapy Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.:

Project Title: Infection, Fever and Host Resistance in Malignant Neoplastic Disease

Principal Investigator: Emil Frei III, M.D.

Other Investigators, Harold Silberman, John P. Utz and John Fahey

Cooperating Units: 1. Laboratory of Clinical Investigation, NIAID
2. Clinical Pathology
3. Metabolism Service, NCI
4. Laboratory of Pathological Anatomy

Man Years (Calendar Year 1959): Patient Days (Calendar Year 1959):
Total: 4
Professional: 2
Other: 2

Project Description:

Objectives: 1. To study the effect of malignant disease on the host as regards resistance to infection.

2. To explore the effects of malignant disease on cellular and humoral factors of resistance.

Methods: Case Material - All patients on the Chemotherapy Service are potential candidates for these studies.

Laboratory - The pathological, serological and bacteriological, biochemical methods are standard except as specifically qualified below.

Major Findings:

1. Acute Leukemia

Our previous studies have indicated that (a) antibody response is normal or at best, only slightly subnormal, in patients with acute leukemia, (b) the phagocytic ability of polymorphonuclear leukocytes is normal in such patients and (c) a marked fall in the absolute polymorphonuclear leukocyte usually precedes infection. Current studies using the Rebuck skin-window technique indicate that at sites of experimental inflammation patients with acute leukemia (a) frequently do not show polymorphonuclear leukocyte mobilization (b) that this ability to mobilize leukocytes correlates highly with susceptibility to bacterial infection and (c) that ability to mobilize leukocytes correlates moderately with the circulating polymorphonuclear cell count.

Part B included Yes

2. Chronic lymphocytic leukemia

The study reported in part in the 1958 Project Report has been completed. The results are as follows: (a) Hypogammaglobulinemia is frequent and tends to be progressive. (b) Response to antigenic stimulation is depressed. The degree of depression correlates well with susceptibility to infection and moderately well with the gamma globulin level. (c) Isohemagglutinin levels are markedly depressed. (d) Delayed skin sensitivity is normal.

Though a retrospective study tended to implicate prednisone in infections in patients with CLL, a prospective controlled study of prednisone has indicated that: (a) the improvement in well-being and rapid and often complete decrease in organ and lymph node size occurs with the use of prednisone, the WBC usually increases. This seemingly discordant effect is unexplained. (b) prednisone did not increase susceptibility to infection in this group of patients.

3. Fever of Undetermined Origin in Neoplastic Disease

A retrospective and prospective study of such fevers has revealed the following: (a) Viral, bacterial and fungus infection is not present (b) Tetracycline, as compared to a placebo, did not influence the course of the fever. (c) Tetracycline adversely affected bacterial flora, particularly as regards resistant staphylococci and monilia. (d) Bacterial infections occurring during therapy were somewhat more frequent and more severe in patients receiving tetracycline.

Attempts were made to demonstrate circulating endogenous pyrogen in man. 300 c.c. of plasma were taken from patients with fever of 39.5° C and returned to them later during afebrile periods. Three such patients were studied, two with fever of malignant disease and one with a fever produced by exogenous pyrogen. We were unable to demonstrate a circulating endogenous pyrogen.

4. Specific Infection

Pseudomonas and clostridial infections have been described (See previous progress reports)

(a) Moniliasis. A prospective study has indicated that
   (1) Thrush occurred in 15% of the patients
   (2) 81% of the episodes of thrush begin during the last 8 weeks of life
   (3) Antibiotics and corticosteroids contribute to susceptibility to monilial infection but are relatively less important than the severity of the disease.
   (4) Nystatin mouth wash (as compared to a placebo) significantly decreased the duration of the thrush.

(b) Cryptococcosis. Disseminated cryptococcus infection with extensive hepatic involvement (not previously described) in the almost complete absence of host cellular response has occurred in 3 patients with lymphoma. These cases are under study.

5. Host response in patients with Hodgkins disease

Early and late patients with Hodgkins disease are being studied as regards
(a) Circulating antibody response
(b) Delayed skin sensitivity
(c) Skin homograft response

The interrelationships of these three factors of host defense as well as their correlation with stage, extent and duration of disease, susceptibility to infection and prognosis are under study.

Significance to Cancer Research

Aside from importance in the clinical management of patients with cancer, the effect of cancer on the host has broad implications as regards the nature of the neoplastic process.

Proposed Course of Project

Most of the above work is clinical with application of standardized laboratory techniques. The clinical work will continue. With the rapid expansion of laboratory techniques and approaches to problems relating to host defense (as represented at NCI by Fahey, Landy and others) it becomes imperative that they be applied to patients.

NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

1. Forkner, C. E., Frei III, E., Edgcomb, J. H., and Utz, J. P. Pseudomonas septicemia: Observations on twenty-three cases. Am. J. Med. 25(6) 877-889, 1958.

2. Boggs, D. R., Frei III, E., Thomas, L. B. Clostridial gas gangrene and septicemia in four patients with leukemia. N. E. J. Med. 259: 1255-1258, 1958.

3. Aronson, S. B., and Shaw, R. Corneal Crystals in Multiple myeloma. A.M.A. Arch Ophth. 61: 541-546, 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Penetration of Anti-tumor Drugs into the Central Nervous System

Principal Investigator: David P. Ball, Charles G. Zubrod

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 2 1/2 0
Professional: 1
Other: 1 1/2

Project Description:

Objectives: The objectives of this project are to quantify the penetration of certain anti-tumor drugs into the central nervous system (CNS) and to attempt to elucidate some of the factors influencing the relationship between dose of drug, plasma level, and concentration of drug in the CNS.

Methods: The pharmacologic technics developed and validated in previous years were used in these studies.

Major Findings: The studies this year progressed along three lines: (1) attempts to find conditions that alter the blood CSF barrier, (2) attempts to delineate the characteristics of drugs which fail to enter CSF (3) attempts to extend the study of the blood CSF barrier to man.

(1) Arterial hypotension and acute cortisone administration failed to alter drug entry into CSF. The blood CSF barrier, measured by our technique, is intact in 5-20 hour old dogs.
(2) Studies in the dogfish demonstrated that sulfanilic acid, even after 96 hours, fails to enter CSF in more than trace quantities. Paraminohippuric acid, on the other hand, if given time, will achieve diffusion equilibrium between blood and CSF. The half-time for this equilibrium is 50 hours. Although sulfanilic acid is often given a dissociation constant of 3.2 for the first hydrogen, it is, in fact, a strong acid and the pK of 3.2 refers to the dissociation of the second hydrogen. Paraminohypparic acid is a true weak organic electrolyte, and about one part in 6000 is undissociated at body pH.

Part B included: YES

The comparison between these two compounds indicates that undissociated drug will penetrate into the spinal fluid; however, in spite of adequate time, dissociated compounds, sulfanilic acid for instance, will not enter spinal fluid in more than trace quantities. Thus the notion that the blood CSF barrier is lipoidal in nature, and consequently is permeable to undissociated, but impermeable to dissociated drug is strengthened (3) Studies on the blood CSF barrier in 16 patients have been completed this year. Antipyrine, sulfadiazine, and paramine hippurate have been the test compounds. CSF was sampled from the lumbar area, instead of the cisternal area as in the dog. These studies have shown that the barrier is essentially the same in man as in the dog. The single difference noted has been that the rate of approach to equilibrium is much slower in man. The half-times for both antipyrine and sulfadiazine are about 5 fold larger in man as compared with the dog.

Significance to Cancer Research: Tumors, both primary and metastatic, frequently develop within the central nervous system. Little information is available in the published literature concerning the penetration of anti-tumor drugs into the CNS. Basic research on this aspect of the pharmacology of anti-tumor drugs is necessary for the rational use of these agents in the treatment of patients with tumors located in and around the brain.

Proposed Course: The following lines of investigation are planned:

1. Further attempts to elucidate some of the basic pharmacologic factors which determine both how much, and how fast drugs get into the CNS and CSF from plasma. These include cerebral blood flow, state of thyroid function, etc.

2. Continued investigation of the comparative pharmacology of CNS and CSF. The technical ease with which the ventricular fluid and brain are obtained in dogfish make this species particularly valuable.

3. Studies in patients with suitable neoplasms will be continued and extended to include the use of anti-tumor drugs.

4. Studies with compounds such as glucose which enter brain and CSF by stereo-specific transport mechanisms.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B.

Rall, D. P., Stabenau, J. R. and Zubrod, C. G. Distribution of Drugs Between Blood and Cerebrospinal Fluid: General Methodology and Effect of pH Gradient. J. Pharmacol. 125: 185-193, 1959.

Zubrod, C. G., and Rall, D. P. Distribution of Drugs Between Blood and Cerebrospinal Fluid in the Various Vertebrate Classes. J. Pharmacol. 125: 193-199, 1959.

Stabenau, J. R., Warren, K. S. and Rall, D. P. Effect of pH Gradients on the Distribution of Ammonia Between Blood and Muscle Brain and Cerebrospinal Fluid. J. Clin. Investigation. 38: 373-383, 1959.

Central No. [illegible]
1. CMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Pharmacology of Antitumor Agents Related to Purine

Principal Investigator: Yi Li Lee

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives: To study the fate and metabolism of certain antitumor agents of the purine group currently in use.

Methods: Standard chemical and biochemical methods were employed.

Major Findings:

1. In the dog, after single i.v. injections of 6-mercaptopurine (6MP), the plasma levels and volumes of distribution were found to support the previous reported findings.

2. The penetration of 6MP through the blood-CSF barrier in the dog after an i.v. injection of the drug was demonstrated spectrophotometrically by examining the ultraviolet absorption spectra of the CSF samples obtained by cisternal puncture. The identity of the drug in the CSF was confirmed by paper chromatography.

3. 6-Thiouric acid was prepared for enzyme inhibition studies.

Significance to Cancer Research: The studies of the metabolic fate of antitumor agents are important because of the light such studies could throw upon the mode of action of these agents. Further, the mechanism of the development of drug resistance could also be partly elucidated through the metabolic studies.

Proposed Course: Continue the isolation and identification of all metabolites of 6MP and 6MP riboside in both responsive and refractory patients.

Part B included: YES

Perk B:

Publications:

1. Lee, T. L., Michael, M. B., Garrett, A. J., and Reid, J. [illegible] 6-thiouric acid, a metabolite of 6-mercaptopurine. J. Am. Chem. Soc., 81: 3039 (1959).

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Metabolism of Folic Acid Antagonists.

Principal Investigators: Ti Li Loo and Vincent T. Oliverio

Other Investigators: None

Cooperating Units: Chemotherapy Service, CMB, NCI

Man Years (calendar year 1959):
Total: 4 2/3
Professional: 1 2/3
Other: 3

Project Description:

Objectives: To study the metabolic transformation of certain folic acid antagonists with a view to elucidating their mode of action.

Methods: Standard chemical and biochemical methods, including ion-exchange chromatography with automatic fraction-acting and recording equipment.

Major Findings:

1. The extraction of methotrexate (MTX) and dichloromethotrexate (DCM) at various pH's by various solvents was studied. It was found that both drugs could be extracted quantitatively into n-butanol from a pH 3 buffer solution.

2. The application of ion-exchange chromatography to the separation and isolation of various folic acid derivatives was intensively investigated. It was discovered that these compounds could be conveniently fractionated and purified by the use of a cellulose ion-exchange system. Mixtures of closely related folic acid derivatives, even those differing only by one functional group, were efficiently resolved into their separate components. This procedure affords folic acid antagonists of a higher purity than hitherto achieved. Several paper chromatographic systems were also developed for the separation of these folic acid antagonists.

Part B Included: NO

with DCM, unchanged drug (10% of dose) and a metabolite (5% of dose) were recovered from the urine of patients. The treatment of the urine of patients on DCM with glucuronidase failed to improve the recovery of unchanged drug and metabolite. No reduced DCM analogous to folinic acid was isolated from the urine of mice injected with DCM. Only the unchanged drug (10% of dose) was recoverable from the urine of a patient on MTX.

4. In connection with the study of the method for the determination of the folic acid antagonists, the oxidation of DCM by potassium permanganate was investigated. At pH 8 the product was 2,4-diamino-6-pteridine carboxylic acid, the structure of which was established by stepwise hydrolytic deamination to the 2,6-dihydroxy homologue, and also by an unambiguous synthesis.

Significance to Cancer Research: The studies of the metabolic fate of anticancer agents are important because of the light such studies could throw upon the mode of action of these agents. Further, the results of such studies may serve as an intelligent guide to the design and synthesis of potential anticancer agents in the future.

Proposed Course:

1. Simplify and improve the present procedure. Continue the isolation and identification of the metabolites of DCM. Continue similar work on MTX.

2. Apply tracer technics to the present investigation in order to study the total drug balance.

3. Apply microbiological technics to evaluate the activity of each metabolite with respect to the action of folic acid

Serial No. NCI-1-227
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Mechanism of Antitumor Action of Purine Analogs

Principal Investigator: Jack D. Davidson

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: The objective of this project is to determine as specifically as possible which biochemical reactions are inhibited by certain purine analogs in cancer cells that are sensitive to these compounds. A corollary objective is to elucidate the mechanisms responsible for inherent and acquired resistance to these agents.

Methods: Radioactive purines, phosphorus and purine analogs were employed with both intact cells and enzyme preparations to study the effects of the drugs upon nucleic acid metabolism in sensitive and resistant mouse and human leukemias.
A methodological advance was made in the development of a ratio recording ultra violet monitor for use in column chromatography. This permitted detection of compounds eluted from ion exchange columns by gradient techniques despite the presence of changing ultra violet absorption intrinsic to the formic acid eluent.

Major Findings: The work initiated last year on 4-aminopyrazolo-(3,4-d) pyrimidine metabolism in man was concluded and is being published (2).
Studies of the mechanism of action of 6-mercaptopurine (6MP) in L1210 leukemia were extended to confirm the findings reported last year. This included dose response studies with both sensitive and resistant L1210 cells to measure the effects of graded amounts of 6MP upon the utilization of hypoxanthine for adenylic and guanylic acid synthesis. All utilization of hypoxanthine was profoundly inhibited by very low concentrations of 6MP in the "resistant" cells while only utilization for adenylic

Part B included: YES

acid moieties was affected in the "sensitive" cells until high concentrations of 6MP were employed. This indicates a block in the conversion of inosinic acid to adenylic acid. The base moiety of the intermediate adenylosuccinic acid was synthesized but despite the use of this as a carrier it was not possible to determine the site of the block more precisely. A final report of all of these 6MP studies is being published (3).

Studies have been initiated to determine whether the mechanisms of 6MP action and resistance determined in the mouse are applicable to human leukemia. For this work 6MP was synthesized with radioactive sulfur. Use of this material with its inherent high analytical sensitivity has necessitated the development of new techniques for isolation of metabolites of the drug. This work is in progress.

Significance to Cancer Research: Some of the most effective antitumor agents thus far discovered are purine analogs. A knowledge of the mechanisms of their action and the reasons for resistance to their action should contribute to the rational development of more effective antitumor chemotherapy.

Proposed Course:

1. Continuation of efforts to determine the mechanisms of action and resistance to 6MP in human leukemia cells.

2. Studies of the metabolism of 6MP in humans.

3. Further efforts to define precisely the significant biochemical action of 6MP responsible for its antitumor activity in L1210 leukemia.

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Part B.

Publications other than abstracts in 1959 attributable to this project:

Nathan, D. G.; Davidson, J. D.; Waggoner, J. G.; and Berlin, N. I. The Counting of Barium Carbonate in a Liquid Scintillation Spectrometer. J. Lab. & Clin. Med. 52: 915-917, 1958.

(This was reported as "In Press" last year)

Shaw, R. K.; Shulman, R. N.; Davidson, J. D.; Hall, D. P.; and Frei, E. Studies with the Experimental Antitumor Agent, 4-Amino-pyrazolo(3,4-d)pyrimidine. Cancer: in press

Davidson, J. D. Studies on the Mechanism of Action of 6-Mercapto-purine in Sensitive and Resistant L1210 Leukemia in Vitro. Cancer Research. in press

1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pharmacology of Riboflavin Analogue

Principal Investigator: Montague Lane

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: A. Clinical. To induce tumor regression and/or clinical riboflavin deficiency in patients with neoplastic diseases through the use of riboflavin analogues.

B. Laboratory. To study the mechanism of action of riboflavin analogues at the cellular level and the transport of flavin nucleotides into cells.

Methods: A. Clinical. Photofluorometry, chromatography, bio-assay and isotope (Fe^{59}) techniques are used

B. Laboratory. Spectrophotometry, bio-assay, isotope and enzyme techniques are used.

Major Findings:

A. Clinical. It has been possible for the first time to induce riboflavin deficiency with a riboflavin antagonist in 2 patients with neoplastic diseases and the findings have been documented photographically and biochemically. No definite evaluation of effects on tumor growth could be made.

B. Laboratory. A number of riboflavin analogues have been tested for their inhibitory effect on growth of L. casei in the presence of riboflavin and its nucleotides. Various action patterns were observed but definite information concerning the sites of antagonism was not developed. Cell entry studies, still in progress, have indicated that flavin adenine dinucleotide probably enters L. casei as an intact molecule.

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Part B included: [illegible]

Significance to Cancer Research:

A. Clinical. Riboflavin analogues have been shown to inhibit rat tumor growth. The development of a regimen which will result in clinical riboflavin deficiency may permit the evaluation of the effects of this deficiency on the growth of tumors in man. Combination chemotherapy studies may also be feasible. In addition, a system is provided for the further elucidation of the physiological role of riboflavin in man.

B. Laboratory. An understanding of the mechanism of action of riboflavin antagonists may aid in the design of more potent chemotherapeutic agents.

Proposed Course:

A. Clinical. Continued clinical trials with galactoflavin and detailed studies of any antitumor effects and biochemical changes will be undertaken.

B. Laboratory. Further studies of cell entry and of isolated enzyme systems will be made.

NIH-(C)1
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Part B.

Lane, M., Petering, H. G., and Brindley, C. O. Synthesis, Pharmacology and Clinical Trial of the Riboflavine Analogue, Iso(6,7-4,5-Dimethyl-2-(2'-Hydroxyethyl) Isoalloxazine, U-6538. J. Nat. Cancer Inst. 22: 346-361, 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

1. [illegible]
2. CP & ET
3. Bethesda

Part A.

Project Title: Studies on Tetracyclines:

I. Chemical Determination of Tetracyclines in Tissues and Tissue Fluids

II. Tissue Distribution of Tetracyclines in Anesthetized Dogs.

III. Interactions of Tetracyclines with Barbiturates, Nucleic Acids, and Proteins.

(This is a continuation of work previously reported under the title: "Fluorochrome Metabolism".)

Principal Investigator: Kurt W. Kohn

Other Investigators: T. L. Loo, D. P. Ball

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2 2/3
Professional: 1 1/3
Other: 1 1/3

Project Description:

Objectives:

I. To develop a method for the determination of tetracyclines in tissues, which would be independent of the problems inherent in bioassays.

II. To determine the volumes of distribution, plasma protein binding, and extent of entry into spinal fluid, red blood cells, and skeletal muscle of tetracycline and demethylchlortetracycline in anesthetized dogs.

III. To study the binding of tetracyclines to small molecules, nucleic acids, and protein.

Methods:

I. Solvent extraction; fluorometry

II. The tetracyclines were determined by fluorometry of their complexes with calcium and barbital, after extraction into ethyl acetate, as described in Part I. Plasma protein binding was determined by a modification of the usual equilibrium dialysis methods. A small volume of isotonic saline in a small bag is dialyzed against approximately 10

Part B included: NO

volumes of heparinized plasma at 37°C. Excellent mixing inside and outside the bag is achieved with a tilting shaker, and equilibrium is established within 4 hours. The plasma is, thus, held at more nearly physiological conditions than in the usual methods.

Customary pharmacologic techniques were otherwise used.

III. Distribution between two liquid phases.

Fluorescence and ultraviolet absorption spectroscopy.

Equilibrium dialysis.

Major Findings:

I. (With Dr. Loo)

Tetracycline was found to be extractable into ethyl acetate in the presence of calcium ions and barbital buffer. Over the optimal pH range of 8.5-9.0, a distribution ratio of approximately 25:1 in favor of the organic phase was observed. The extraction did not take place if either Ca^{++} or barbital were omitted from the system; nor could Ca^{++} be replaced by Mg^{++}, or barbital by other buffers. The extraction was attributed to the formation of an uncharged molecular complex consisting of tetracycline, Ca^{++}, and barbital. The complex is strongly fluorescent and can be measured in the ethyl acetate phase in concentrations as low as 10^{-7}M. Methods were readily developed for determinations over the usual therapeutic range of concentrations in plasma, red cells, spinal fluid, urine, and bile. Attempts to apply the method to tissues were at first unsuccessful. This was believed to be due to the presence of phosphates which, under the alkaline conditions of the extraction, form calcium phosphate precipitates, which adsorb tetracycline. The difficulty was finally overcome by the use of Fe^{++} to precipitate phosphates at pH 8. Excess Fe^{++} was then precipitated with 10^{-3} M; the latter did not interfere with the extraction step. This modified method was successfully applied to determinations in skeletal muscle, liver, kidney, and tumor tissues. The drug was, in each case extracted from the tissue homogenates with dilute HCl with recoveries of 80-100%. The blank readings were generally very low, and no significant false positive reactions have been found. The method appears to be highly specific for certain tetracyclines. Such small changes in the molecule as occur in 4-epi-, desdimethylamino-, and anhydro-tetracyclines are present or greatly reduce, the formation of extractable fluorescent complexes under the conditions employed. The method has also been successfully used to determine demethyl-chlor-tetracycline in tissues and body fluids. Little work was done with chlor-and oxy-tetracycline because of their lower stabilities.

II. (With Dr. Rall)

Plasma concentrations of tetracycline (TC) and dimethylchlortetracycline (DMCT), after single i.v. doses, followed first-order disappearance curves after 2 hours following drug administration. The plasma half-times were 3.1-4.4 hours for TC and 5-14 hours for DMCT (ranges for 3 dogs on each drug). Extrapolation to zero time of the linear semi-logarithmic plots, permitted the calculation of volumes of distribution; these were 0.58-0.64 l/kg for TC and 1.1-1.4 l/kg (liters per kg body weight) for DMCT (ranges for 3 dogs on each drug). Plasma protein binding studies, by equilibrium dialysis at 37°, indicated 50% binding of DMCT and approximately 35% binding of TC. In terms of the serum concentrations of free drug, the volumes of distribution were, therefore, approximately 0.94 and 2.5 l/kg for TC and DMCT, respectively. These results indicate that DMCT and, to a lesser extent, TC are concentrated in some extravascular compartment of the body.

This picture was supported by measurements of concentrations in skeletal muscle, which, after 6 hrs., were 1.2-1.5 and 2.3-2.5 times the (total) plasma concentrations for TC and DMCT respectively. In some of these experiments, constant intravenous infusions of drug were given, in addition to a loading dose, in order to maintain relatively constant plasma levels.

During the course of these experiments, both drugs entered the spinal fluid in concentrations equal to approximately 20% of the concentrations free in plasma. Equilibration between plasma and spinal fluid was probably not achieved, and experiments are in progress to determine the concentration ratios after allowing several days for equilibration.

Considerable amounts of these drugs were found to be associated with the red blood cells. Equilibration between red cells and plasma took place within 15 minutes in vitro, and the rate of drug disappearance from red cells closely followed the plasma disappearance curves in vivo. Because of variability in the red cell-to-plasma concentration ratios in different experiments, the effects of (Ca^{++}) and pH were studied, using washed human red cells suspended in isotonic saline. It was found that a 2-fold and 4-fold concentration in red cells took place in the case of TC and DMCT, respectively, at pH7. At pH 6.5-7.5, Ca^{++} (in concentrations comparable to those found physiologically) markedly reduced the amount of either drug associated with cells, while, at pH 7.8 and above, a second effect of Ca^{++} became apparent which resulted in increasing the amounts of drug bound to the cells. Although the interpretation of these results is not yet clear, they do point out the importance of considering divalent metal ions in trying to understand the distribution of these drugs.

III. The formation of complexes of tetracycline (TC) with Ca++ and other divalent metals is well known. Complexation with barbiturates and with β-diketones has not been previously described to our knowledge. Distribution between ethyl acetate and water at pH9 was found to be a convenient system for studying these complexes. TC concentrations were determined in each phase, after equilibration. From the results of these studies, it was inferred that TC forms an unionized complex with Ca++ and barbital. Similar complexes were observed with Zn++ and, to a much lesser extent, with Mg++, but Mn++ failed to form extractable complexes. Phenobarbital and pentobarbital were more potent than barbital in causing extraction. Methyl substitution of one of the nitrogens in the barbiturate nucleus did not change its potency in this regard, but substitution of both nitrogens rendered the molecule inert. Diphenylhydantoin and oxazolidinediones had no complex-forming activity, nor did a variety of other compounds. It was later found that some β-diketones also form extractable complexes with TC and Ca++. Cyclic β-diketones, however, were effective, indicating that a geometrical form able to chelate is required, and suggesting that the nature of the binding is different than with barbiturates.

Attempts were made to detect changes in fluorescence and ultra-violet absorption, which might accompany complexation. An increase in fluorescence intensity was noted in aqueous solution, attributable to the formation of a barbital complex. No significant changes in absorption spectra or optical density were observed, however, suggesting that the barbiturate may not bind to the chromophoric part of the tetracycline molecule.

Equilibrium dialysis studies were carried out to determine whether tetracyclines bind to nucleic acids and proteins. The most striking results were obtained with DNA. Binding was observed in the presence of Ca++, Mg++, or Zn++. There was no binding in the absence of divalent metal ions. Heat denaturation of DNA increased its ability to bind tetracyclines. There was much less binding to RNA. Human serum albumin bound tetracycline to a small extent. The degree of binding was greatly increased by Zn++, but not by Ca++ or Mg++.

Significance to Cancer Research: Tetracyclines exhibit the ability to concentrate selectively in certain tissues, including some tumor cells. This binding may be a tool for improving our knowledge of the composition or structure of tumor cells under various conditions.

An understanding of the factors which govern the cellular distribution of these drugs may be of general value in chemotherapeutic problems. The tetracyclines are also of interest because of their antibiotic properties. Elucidation of the mechanism in this instance of selective cellular toxicity would be of vital interest.

The present studies on the complexing properties of tetracyclines are preliminaries aimed at these goals.

Proposed Course: Further work on the binding of tetracyclines in cells is planned, involving

(a) in vivo studies of drug distribution in tissues in dogs and tumor-bearing rodents.

(b) studies of binding to red cells, and tissue homogenates under varying conditions of pH and divalent metal ion concentration.

(c) quantitative studies of binding to purified tissue components, i.e. proteins and nucleic acids.

(d) cytochemical studies on the composition of the fluorescent material which persists in tumors after tetracycline administration.

Serial No. NCI-1-232
1. GMB
2. CP & ET
3. Bethesda

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Individual Project Report
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Part A.

Project Title: Pharmacology of Drugs Influencing the Response to Radiomimetic Drugs and Radiation

Principal Investigator: Margaret G. Kelly

Other Investigators: Roger W. O'Gara, Ti Li Lee

Cooperating Units: NCI Laboratory of Pathology

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To study the mechanism of protective effect of AET on toxicity of nitrogen mustard (HN2) in normal and tumor-bearing mice and rats.
To determine localization of S-35 and C-14 labelled AET in tissues and within cells by application of autoradiogram techniques, and to compare this with distribution of other sulfhydry compounds.
To evaluate protective effect against radiomimetic drugs and radiation of other classes of compounds (in addition to mercapto-alkylguanidines) and to explore possible advantage of combinations of protective agents.

Methods: Usual pharmacologic, hematologic, chemical, biologic, histopathologic, and radioactive tracer techniques.

Major Findings:

1. Pretreatment with AET had no effect on the decrease in peripheral blood count produced by HN2 in normal mice, rats, and dogs. In rats bearing subcutaneous implants of Dunning leukemia, the associated sharp increase in peripheral leukocyte count was prevented or minimized to about the same degree in rats pretreated with AET as in rats given HN2 only.

2. The Dunning leukemia in rats is more sensitive to nitrogen mustard therapy than any of the tumors in mice which we have used. An increase in survival of rats bearing this tumor can be obtained at relatively non-toxic dose levels of HN2, thus permitting a more precise evaluation of any dose reduction effect of pretreatment.

Part B included: YES

with AET. So far as can be judged from examination of survival data (statistical evaluation has not been made as yet), AET does not minimize the tumor-damaging effect of low doses of HN_2, and at higher dose levels of HN_2, pretreatment with AET results in a therapeutic advantage by protecting the host from the toxic effects of nitrogen mustard.

3. 3. Tolerance to the lethality of AET could not be demonstrated in mice after repeated daily, every 2 days, or weekly administration of non-lethal doses of AET.

4. See O'Gara (Lab. Path.) for results of autoradiographic study with S^{-35} and C^{-14}-labeled AET and thiourea, and S^{-35}-labeled Na_2SO_4.

5. (With Dr. Loo) Studies have been started to determine the effect on toxicity and tumor damaging action of radiomimetic drugs and radiation, of compounds which generate free radicals. To date, t-butyl perbenzoate, di-t-butyl peroxide, benzoyl peroxide, and a hydroxylating reagent composed of ferrous sulfate, hydrogen peroxide, ascorbic acid, and EDTA have been tested against HN_2. The hydroxylating reagent did not decrease the toxicity of HN_2 and may have increased it. The other 3 compounds, however, gave effective protection against an LD_{50} of HN_2 when given 30 to 60 minutes before the nitrogen mustard.

Significance to Cancer Research: Chemical protection against systemic toxicity of alkylating agents or radiation without comparable protection of tumor against carcinolastic action would result in a therapeutic advantage.

Proposed Course of Study: Comparative study of urinary excretion products of C-14-labeled AET in dogs and in mice and rats in the hope of getting some information or understanding of the underlying basis of the species differences in toxicity.
To complete the study of cellular distribution of labeled AET in mice by use of microautoradiograms.
To evaluate the protective effect on toxicity and tumor-damaging action of HN_2 and radiation of free radical generating compounds singly and in combination with AET.

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Calendar Year 1959

Part B.

Actions of S, 2-aminoethylisothiuronium Br. HBR (AET). Toxicity and Protective Effect against Nitrogen Mustard Toxicity, by Margaret G. Kelly, David P. Rall, Glenn E. Trivers, Roger W. O'Gara, and Charles G. Zubrod, J. Pharm. & Exp. Ther. In press.

Serial No. NCI-1-233
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemical Carcinogenesis

Principal Investigators: Margaret G. Kelly and Roger W. O'Gara

Cooperating Units: Lab. Pathology, NCI

Man Years (calendar year 1959):
Total: 1.0
Professional: 1/2
Other: 1/2

Project Description:

Objectives: To determine the effect of carcinogenic hydrocarbons in newborn mice.

Methods: Usual biologic and histopathologic techniques.

Major Findings:

1. We have confirmed the results reported last year on the induction of lung tumors in General Purpose (Swiss) mice following subcutaneous injection of newborns with either 0.06 mg of 1,2,5,6-dibenzanthracene or 0.1 mg of 20-methylcholanthrene in olive oil. Even considerably lower dose levels than those reported above produce a high incidence of multiple lung tumors in this strain. After subcutaneous injection of either 0.02 mg of dibenzanthracene or 0.03 mg of methylcholanthrene to newborn General Purpose mice, multiple lung adenomas were found in 50-80% of the animals killed in 8 weeks, and in 90-100% of the animals killed in 16 weeks. Two lower dose levels of dibenzanthracene (0.0067 and 0.0022 mg) induced lung tumors in 25% of the animals killed in 8 weeks, and in 55 and 33% respectively, of those killed in 24 weeks. A lower dose of methylcholanthrene (0.01 mg) induced lung tumors in 25% of the mice killed in 8 weeks, 67% of those killed in 16 weeks, and 100% of those killed in 24 weeks. In general, the incidence of mice with lung adenomas and the number of tumors per affected mouse increased directly as the dose was increased and the time before sacrifice was lengthened.
In addition to fibrosarcomas (total incidence ca. 10% at 16 weeks), some other pathologic lesions have been observed. Four mice given methylcholanthrene subcutaneously as newborns developed leukemia (in 9, 12, 14, and 21 weeks, respectively), 1 developed a thymic

Part B included: NO

lymphoma, 1 had a lesion of the pancreas. One of the mice given dibenzanthracene developed an intraductal papilloma of the mammary gland. Histologic sections of mice killed after 21 weeks are not yet available for study, and will be reported later.

2. An evaluation has been made of the susceptibility of newborn C3H and C-57Bl mice to subcutaneous administration of carcinogens because adult mice of these strains are relatively resistant to lung tumor induction by intravenous as well as subcutaneous administration of carcinogenic hydrocarbons.

A. C3H Mice:
Of 50 newborn C3H mice given 0.06 mg dibenzanthracene subcutaneously, lung tumors were found in 12% of the mice killed in 16 weeks, in 44% at 18-23 weeks, and in 74% of the mice killed in 24-27 weeks. The incidence of subcutaneous tumors in this strain was 24%, the earliest tumor being noted in 8 weeks. One mouse had cataracts of both eyes.
Of 30 newborn C3H mice given 0.1 mg methylcholanthrene subcutaneously, lung tumor were found in 33% of mice killed in 16 weeks, and 93% of those killed in 24-28 weeks. The incidence of subcutaneous tumors was 40%. Four mice had papillomas of the skin, and 2 had hepatomas.

B. C-57BL Mice:
A single lung tumor nodule was found in 2 of 22 mice killed in 12 to 24 weeks after 0.06 mg dibenzanthracene, and in none of 17 mice killed in 12-27 weeks after methylcholanthrene. The incidence of subcutaneous tumors has been 10% in the dibenzanthracene group and 35% in the methylcholanthrene group. Additional pathologic lesions have developed in the mice given methylcholanthrene: one mouse had a thymoma, and another had leukemic implants in lungs, thymus liver, spleen lymph nodes, and kidney. In 5 of 10 mice remaining 27 weeks after injection of methycholanthrene, a deformity of the chest has recently been noted. The xiphoid process is protuberant, and the sternum is depressed and concave.

3. A comparison has been made of the relative susceptibility to lung tumor induction of mice injected as newborns, and of mice injected at 1, 3, and 6 weeks of age. General Purpose mice were used in this study, and were given either 0.06 mg dibenzanthracene of 0.1 mg methylcholanthrene subcutaneously. Eight weeks after injection of either carcinogen, the incidence of lung tumors was about 50% in the newborns, and 10-20% in the animals injected at 1, 3, or 6 weeks of age. By 16 weeks, the incidence in the 1 week old group was comparable to that in newborns (86% and 83%, respectively) while the incidence in the older age groups was still low. By 24 weeks, all of the mice injected at 1 week of age had lung tumors, while in those injected at 3 weeks of age, 17% of the dibenzanthracene group, and none of those given methylcholanthrene had developed lung tumors. In addition, the number of tumors per affected mouse decreased as the age at which the carcinogen was given was increased (average of 1-3 per affected mouse in the 3 and 6 weeks of age groups).

4. Effect of intraperitoneal administration of these carcinogens has been studied. Newborn General Purpose mice were injected intraperitoneally with either 0.015 mg of dibenzanthracene of 0.025 and 0.05 mg of methylcholanthrene contained in 0.05 ml of olive oil. One of 3 mice killed 24 weeks, and 31 of 34 killed between 35 and 42 weeks had multiple lung tumors. In addition, 3 of the mice killed in 41 weeks had hepatomas, 1 had a hemangioma of the liver, 2 had hepatic cysts, 2 had renal cysts, one had a lesion of the parotid gland, another a papilloma of the skin, and the ovarian tissue of 3 mice was largely destroyed and the ovary filled with large channels dilated with blood.

Significance to Cancer Research: The greater susceptibility of newborn and very young suckling mice to chemical carcinogens may be of importance in setting safe standards for the human population. Furthermore, the use of newborns offers the possibility of a more sensitive system with which to determine the carcinogenicity of food additives, etc. Carcinogen induced tumors would be useful in chemotherapy studies.

Proposed Course of the Project:

To evaluate the susceptibility of newborn mice to a few other carcinogens.

To determine whether subminimal dose levels of 2 or more carcinogens have an additive effect and would result in lung tumor induction in newborn mice.

To examine the possibility of using tumors induced in newborn mice as a tool in evaluating the relative anti-tumor activity of drugs of interest in cancer chemotherapy.

To determine the effect of carcinogens administered to pregnant mice by intravenous or intraperitoneal route on the incidence of tumors in their progeny.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pharmacology of Alkylating Agents

Principal Investigator: Montague Lane

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2.0
Professional: 0.5
Other: 1.5

Project Description:

Objectives:

A. Laboratory. To study the pharmacological effects and anti-tumor activity of various alkylating agents in mice and rats bearing established tumors so as to develop information which might facilitate the clinical trial of new agents.

B. Clinical. A. To submit these agents to preliminary pharmacological trial in patients with advanced neoplastic diseases.

B. To correlate the effects of alkylating agents on ferrokinetics with various hematologic parameters.

Methods:

A. Laboratory. Usual pharmacologic, hematologic, biologic and histopathologic techniques.
B. Clinical. Radioactive iron tracer studies (including external body counting), chemical determinations of iron, and usual clinical techniques.

Major Findings:

A. Laboratory.

1. The toxicity of cyclophosphamide has been defined in mice and rats. Unlike HN2, the drug had little effect on gastro-intestinal epithelium. It invoked a marked megaloblastic hyperplasia in the spleen and bone marrow 5-10 days after administration. The LD50 for mice was about 425 mg/kg and for rats was 150 mg/kg.
2. Cyclophosphamide has been shown to be more effective than other alkylating agents and methotrexate in prolonging the survival of mice with advanced L-1210 leukemia. It was

Part B included: YES

similarly effective against the 6-mercaptopurine resistant cell-line of L-1210 leukemia (which is also 6-MP resistant).
3. The agent has been shown to be considerably more effective on a once weekly treatment schedule than on other regimens.
4. When given on such a schedule, cyclophosphamide has been shown to produce marked increases in survival of animals bearing well established tumors(lymphoma L-2, carcinoma 261-6, Dunning leukemia and Dunning lymphosarcoma), and to be far more effective than nitrogen mustard.

B. Clinical:
1. A study of the pharmacology of uracil mustard was completed in cooperation with Drs. Hall and Colsky of the Eastern Solid Tumor Chemotherapy Group. The design of this study was based upon information previously obtained in animal and clinical studies at NCI. The systemic toxicity of oral doses of uracil mustard in man was found to be similar to that of intravenous doses of nitrogen mustard, i.e., nausea, vomiting, anorexia and bone marrow depression. The following doses were found to be tolerated for 6 weeks with minimal toxicity: 0.015 mg/kg daily; 0.2 mg/kg once weekly; 0.45 mg/kg as a single dose. In this study, objective tumor regression was seen in some patients with Hodgkin's disease, lymphosarcoma, and chronic lymphocytic leukemia.
2. Ferrokinetic studies were completed in one patient and are in progress in a second patient. Uptake of Fe^{59} by red cells was shown to be impaired 4 hours after a dose of nitrogen mustard.

Significance to Cancer Research:

A. Laboratory. 1. The studies with uracil mustard and with cyclophosphamide have emphasized the importance of evaluating the performance of drugs in animal tumor systems on the basis of optimum schedules of treatment.
2. The results of the studies with cyclophosphamide in mouse leukemia gave encouragement to the evaluation of this alkylating agent in adult and childhood human leukemia, in which conditions it has produced some "complete remissions".
3. The consistently greater anti-tumor effects obtained with intermittent treatment in animals has provided a pattern for the design of clinical programs which compare the effects of different schedules.

B. Clinical. 1. The qualitative and quantitative aspects of the toxicity of a chemotherapeutic agent must be determined prior to extensive clinical trial.
2. The ferrokinetic studies may provide insight into the evolution of hematologic changes which are induced by alkylating agents.

Proposed Course:

A. Laboratory. Completion of studies of effects of cyclophosphamide and of mechanism of action of this agent.

B. Clinical. Continuation of ferrokinetic studies currently in progress with nitrogen mustard and other alkylating agents.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B.

Lane, M.: Preliminary report of animal studies with cytoxan (cyclophosphamide). Cancer Chemotherapy Reports, No. 3, 1-6, 1959.

Lane, M.: Some effects of cyclophosphamide (cytoxan) on normal mice and mice with L-1210 leukemia. J. Nat. Cancer Inst. 23, Dec. 1959.

Lane, M.: The antitumor activity of 5-bis-(2'-chloroethyl)-aminouracil (uracil mustard). Cancer Research In press.

2. CP & ET
3. Bethesda

Part A.

Project Title: Preclinical Toxicology of Drugs Potentially Useful in Cancer Chemotherapy

Principal Investigator: David P. Rall

Other Investigators: Margaret G. Kelly

Cooperating Units: CCNSC - NCI
Hazleton Laboratories, Falls Church, Va.

Man Years (calendar year 1959)
Total 1/3
Professional 1/3
Other 0

Project Description:

Objectives: The objectives of this project are to study, in detail the quantitative and qualitative aspects of the toxicity of drugs potentially useful in cancer Chemotherapy in order that new drugs may rapidly but safely be brought up to the point of clinical trial.

Methods: Standard toxicological methods are employed.

Major Findings: The studies this year have been concerned with cytoxan, a new alkylating agent, 6-mercaptopurine, 4 aminopyrazolo pyrimidine, nitrogen mustard, a combination of various doses of 6-mercaptopurine and amethopterin, and actinomycin, as well as further studies on derivatives of amethopterin and its halogenated derivatives and E-73. Studies on the effect of age and sex on the toxicity of E-73 were extended. Castration of male rats increased their susceptibility to E-73 5-10 fold and brought it into the range of normal female. There was no difference in E-73 toxicity between male and female dogs. The pulmonary lesion observed in patients who received E-73 was reproduced in the dogs. Studies of nitrogen mustard and of 6-MP confirmed in general previously recorded toxicological data. Of particular interest is the finding that 6-mercaptopurine in the dog increases serum transaminase activity at sub-lethal levels.
The demonstration of the effectiveness of cytoxan against rodent tumors when this alkylating agent was given at widely spaced intervals by Dr. Lane of this Service led to a study of the toxicology of cytoxan using widely spaced dose schedules in dogs and rats. The advantage, in terms of administration of more drug, of the less frequent drug administration schedules was clearly shown. Of particular interest, also, is the possibility that in the rat 10-14 days following a sub-lethal dose of cytoxan (and also amethopterin) there is a period of decreased susceptibility to the

Part B included: YES

the lethal action of the drug in question. Such an observation could serve as the basis for an explanation of the increased therapeutic benefit of certain dose schedules.
One potentially important study with the amethopterin derivatives has been the comparison of the toxicity of these agents when given by a constant infusion over a 48 hour period against repeated daily administration. In dogs the constant 48-hour infusion is more toxic than is daily administration for 14 days.

Significance to Cancer Research: The drugs currently useful in the treatment of human neoplasms must be given at near toxic doses to achieve therapeutic results. Therefore even small differences in toxicity related to sex, age, route of administration and dose schedule, can be of considerable importance in clinical chemotherapy since this may lead to larger doses safely given to man. Information for preclinical toxicology is quickly made available to the Cooperative Groups of the CCNSC.

Proposed Course: It is planned that this project will be continued along the same lines as above. Drugs showing promise in Dr. Goldins quantitative animal tumor studies, or drugs obtained from CCNSC will be put into this toxicological appraisal before deciding on the dose, route and schedule of administration in man.

Part B.

Shaw, E. E.; Shulman, P. M.; Davidson, J. D.; Hall, N. P.; and Pratt, E. Studies with the Experimental Antitumor Agent, 4-Aminopyrazol (3,4-d)pyrimidine. Cancer: In press.

Serial No. NCI- 1-236
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of the Hypermetabolic State in Malignancy on Metabolism of Thyroxine and Hydrocortisone.

Principal Investigator: Leonard Garren

Other Investigators: None

Cooperating Units: Jack D. Davidson, No. NCI-1-227; Mortimer Lipsett, No. NCI-2-803 el

Man Years (calendar year 1959):
Total : 1
Professional: 1
Other : 0

Project Description:

Objectives: To observe if the hypermetabolic state found in certain malignant diseases influences the metabolism of thyroxine and hydrocortisone similarly to that observed in the hypermetabolism due to hyperthyroidism.

Methods: Labeled I^{131} thyroxine and C^{14} cortisol are injected into hypermetabolic patients and the rates of disappearance of these hormones are followed. Subsequently, after the malignancy is treated and the patients are eumetabolic the studies are repeated.

Major Findings: The studies are in progress and sufficient data are not yet available.

Significance to Cancer Research: Since the hypermetabolic state of malignancy at least superficially resembles that found in hyperthyroidism, an investigation into its effect on specific parameters which hyperthyroidism is known to influence should permit a better understanding of this metabolic abnormality

Proposed Course:

1. Further studies as outlined above until sufficient data are accumulated.

2. To investigate the rate of metabolism of these hormones in hyperthyroidism in order to establish that the techniques used in these experiments are valid.

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Serial No. NCI-B-237
1. GMB
2. CP & ET
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemical and Pharmacological Studies of Human Neoplasms in Tissue Culture

Principal Investigator: William C. Mahler

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description

Objectives:

1) To develop practicable and reliable techniques for growth and measurement in vitro of cells from human biopsy material.

2) To employ these methods
   a) for studying the mechanism of action of therapeutic agents
   b) in developing a test for the sensitivity of a patient's tumor to selected therapeutic compounds.
   c) as a means of studying biological and biochemical variation of human neoplasms.

Methods: Cells are handled as single cell suspensions and grown as monolayer sheets or clones, on glass, in defined media, supplemented when necessary with serum or other biologicals.

Major Findings: Work during the first half year of this project has involved only setting up methods for growth and measurement of standard cell lines.

Significance to Cancer Research: The measurement of growth and the metabolism of normal and neoplastic cells in vitro provides a means of examining the conditions required for maximal growth rates in normal and neoplastic tissues and the conditions under which neoplastic cells may be selectively killed or inhibited.

Part B included: NO

So-called plating techniques provide a means of analyzing the kinetics of drug action on pure cell strains or mixtures of cell types. Such studies should afford insights into the mechanisms of action of therapeutic drugs and may afford a basis for modification of treatment schedules in man. When combined with histochemical or nutritional techniques these methods should make possible the analysis of cellular population dynamics during the natural history of neoplasms and following their treatment.

Proposed Course:

1. Study of the action of selected drugs on some established cell strains in order to develop methods and gain a knowledge of the kinetics of drug action.

2. Make attempts to grow new human cell lines from selected human tissues, with special attention to establishing methods for obtaining a high recovery of viable cells from biopsy material (including blood culture).

3. Study histochemical methods for identifying specific cell types in cultures.

4. Attempt "clinical tests" of the sensitivity of biopsies of human neoplastic tissues to selected chemotherapeutic agents.

[illegible]

Individual Project Report
Calendar Year 1959

Part A:

Project Title: Clinical Investigations of Malignant Skin Diseases and of Benign Conditions Related Thereto.

Principal Investigator: E. J. Van Scott, M. D.

Other Investigators: Richard P. Reinertson, M. D., Arthur Z. Eisen, M. D.

Cooperating Units: Chemotherapy Service, NCI

Man Years (calendar Year 1959): Patient Days (calendar year 1959):

Total:	2
Professional:	2
Other:	0

Project Description: Continuation of Z-7-708(c) of 1958

Objectives: To study malignant processes in the skin and to carry out investigative therapy of cutaneous malignant conditions; to study benign hyperplasia of the epidermis in relation to malignant hyperplasia of the skin and other organs.

Methods Employed:

Mycosis fungoides: This disease is treated with chemotherapeutic drugs given systemically and with electron or x-radiation when indicated, and is further studied and treated by other means derived from observations on the immunologic behavior of the disease.

Basal cell carcinoma: Severe cases are treated with chemotherapeutic drugs given systemically, with biopsy, and with excision. The nature of the tumor is investigated in the patient by studying changes in the tumor occurring in response to locally administered agents and by alterations in the stromal environment of the tumor.

Part B included: Yes /X/ No /__/

Psoriasis: Severe cases are treated experimentally with chemotherapeutic drugs either applied topically to the skin or given systemically. The nature of the disease and the cutaneous lesion is studied in the patient.

Patient Material: Patients of the Dermatology Service, NCI

Major Findings:

Mycosis Fungoides: Beneficial but temporary response of the disease has been observed following chemotherapeutic drugs, x-rays and electron radiation; no new data has been collected relative to these forms of therapy.

A single patient with a previous diagnosis of mycosis fungoides has been observed to be totally free of this disease for over two years, the original disappearance of cutaneous lesions having been associated with a rather marked drug eruption. Whole blood from the patient has been transfused on two occasions into another patient with mycosis fungoides; no detectable effects on the recepient's disease have occurred from this procedure. Attempts have been made to sensitize patients with M. F. to a number of drugs. In one patient this has been accomplished with resultant skin eruption, marked eosinophilia, fever and pulmonary infiltration suggestive of Loeffler's syndrome. Of five cutaneous tumors observed in this patient, three disappeared clinically, one underwent marked reduction in size, and one remained unchanged. (Remaining cutaneous tumors treated with x-ray.

Basal Cell Carcinoma: Patients with sufficiently severe basal cell cancers have not been available for systemic therapy with chemical agents during the past year.

Various lines of study on epidermal and tumor growth have been pursued in patients with this disease, as well as with other diseases. Auto-transplantation of epidermal tissues free of donor site stromal connective tissue indicates that a tissue may be either incapable of survival in a new environment, or that it assumes the characteristics of epidermal tissue normally resident in the transplant site. Thus, for example, oral mucous membrane upon transplantation into the superficial corium of skin undergoes modification like epidermis, but upon implantation into the deep dermis is incapable of surviving. Similar manipulation of basal cell tumor tissue has given preliminary indication that this tumor cannot survive in the deeper dermis unless accompanied by the stromal connective tissue of the donor site.

Psoriasis: Many chemical agents used in cancer chemotherapy have been applied topically to lesions of psoriasis. Complete clearing of lesions, clinical and histologic, has occurred within one week following twenty-four hour applications of nitrogen mustard, podophyllin, colchicine, mercury, and tar. Cleared areas tend to relapse within one to several weeks. No local response to Methotrexate has been observed, although this drug markedly clears the skin of lesions when given systemically.

Methotrexate, Actinomycin D, 5Fluorouricil and colchicine have been administered systemically to patients with psoriasis. Clearing of the skin has occurred following all these chemical agents.

Serial histological studies of lesions have indicated that the lesion of psoriasis may be characterized as hyperplasia of the epidermis, that hyperplasia is slowed with cytotoxic agents allowing normal keratinization to take place.

Significance to Cancer Research: Project directly concerned with study of, and therapeutic influences on, cancer and related conditions.

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Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Cramer, L. M., Waite, J. H., Edgcomb, J. H., Powell, C. C., Touhy, J. W., Van Scott, E. J., and Smith, W. M. Burn Following Accidental Exposure To High Energy Radiation, Ann. Surg. 149: 286-293, 1959.

2. Van Scott, E. J. and Reinertson, Richard P. Reinertson: Morphologic and Physiologic Effects of Chemotherapeutic Agents in Psoriasis, J. Invest. Dermat. (in press).

Honors:

1. Member of Dermatology Service (E. J. Van Scott) elected to Board of Directors, Soc. for Investigative Dermatology, 1959-1964.

2. Member (E. J. Van Scott) elected to membership, Amer. Dermatological Assoc.

Serial No. C-7-728
1. General Medicine Branch
2. Dermatology Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Structural and Physiological Characteristics of Normal and Pathological Epidermis and Hair Roots.

Principal Investigator: E. J. Van Scott, M. D.

Other Investigators: Richard P. Reinertson, M. D., Robert G. Crounse, M. D. (Research Fellow)

Cooperating Units: All clinical units of NCI, to some degree

Man Years: (calendar year 1959) Patient Days (calendar year 19

Total:	3
Professional:	1
Other:	2

Project Description: Continuation of C7-728(c) of 1958

Objectives: To investigate structural and physiological characteristics in epidermal and supporting tissues in pursuit of understanding wide ranges of growth activity of epidermal tissues and regulatory factors thereof.

Methods Employed: Tissues are studied histologically and from reconstructions of specific structures. Total number of cells and mitotic figures counted microscopically, volume of specific tissue structures calculated from microscopic measurements, geometric relationships .

Further comparisons are made on the basis of structural changes, particularly changes in hair roots, following ionizing radiation or drugs.

Normal scalp hair roots are cultured in Algire millipore filter chambers implanted in the abdomen of mice to study growth regulating factors.

Patient Material: Tissues studied or used are from hospitalized patients and out-patients of Dermatology Service and other clinical services of N.C.I. Differentiation of epidermal cells and regulatory influences on keratinization are studied in patients by manipulative auto-implantation of epidermal tissue.

Major Findings: The essential dependence of hair growth on the connective tissue dermal papilla has been further elucidated in humans from histological studies of geometric relationships within the hair root. Mitotic activity of the germinative matrix is directly proportionate to the number of cells in the papilla, 1 mitosis normally and uniformly present per 8 papilla cells. Permanent baldness in alopecia areata has been found to be associated with destruction of the dermal papilla.

Mitotic activity, decreasing in successively higher levels of the hair bulb, is found to further be a function of the area of the dermal papilla to which the matrix cells are exposed and to the transverse thickness of the matrix at each level.

Reversible damage to the growing hair roots of the human scalp has been found following a number of chemotherapeutic agents, including methotrexate, di-chloro-methotrexate, 5-fluorouracil, actinomycin D, thio-tepa, colcemid, cytembena and vinca leukoblastin. Damage to hair roots has been found to be a sensitive indicator of systemic effects of these drugs, occurring with equal or greater frequency than changes in WBC, platelets, or reticulocytes.

The process of keratinization of epidermis has been studied in humans by imbedding isolated epidermis intradermally. Differentiation of cells and keratinization occurs in the central areas of masses of implanted cells, suggesting several mechanisms by which keratinization and differentiation may occur.

Significance to Cancer Research: The studies are primarily concerned with cellular reproduction, destruction or quiescence, phenomena primary in studies of cancer.

Proposed Course of Project: Continuation along lines presently as indicated in the foregoing.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part D: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Van Scott, E. J., Evaluation of Disturbed Hair Growth in Alopecia Areata and Other Alopecias. Ann. N.Y. Acad. Sci. (in press).

2. Crounse, R. G. and Stengle, J. M., Influence of the Dermal Papilla on Survival of Isolated Human Scalp Hair Roots in An Heterologous Host. J. Invest. Dermat. 32: 477-479, 1959.

Serial No. C-7-730
1. General Medicine Branch
2. Dermatology Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The Biochemistry of Normal and Pathological Epidermis.

Principal Investigator: Simon Rothberg, Ph. D.

Other Investigators: Eugene J. Van Scott, M. D., Robert G. Crounse, M. D. (Research Fellow)

Cooperating Units: None

Man Years (calendar year 1959): Patient Days (calendar year 1959)
Total: 3
Professional: 2
Other: 1

Project Description: Continuation of C7-730 of 1958

Objectives: To study the biosynthesis of the major protein, keratin, in normal and abnormal epidermis. Knowledge of this process will permit examination and comparisons as to the nature of the protein synthesized in normal and diseased states.

Evaluation of the differences between the major keratin protein (or proteins) of normal and pathological human epidermis in order to determine whether an abnormal keratin does or does not exist.

Estimation of the arginase content of the dissected component of the human hair root.

Description of the Krebs-Henseleit urea cycle in human epidermis.

Part B included Yes /X/ No /___/

Methods: Solubilization, isolation and purification of the major protein fractions present in normal and pathological epidermis are made. Comparisons are made of the fingerprint patterns produced by the individual proteins in order to detect differences in these proteins which may otherwise be undetectable.

The peptides obtained by trypsin and chymotrypsin hydrolysis of the purified protein fractions are separated utilizing, first, one dimensional descending chromatography and then high voltage electrophoresis. The location of the peptides is visualized by the ninhydrin reaction with the peptides. "Fingerprint" patterns of the protein fractions are compared and the peptide differences between different pH fractions of the same tissue (epidermis, psoriasis scale, or ichthyosis scale)or between similar pH fractions of different tissues revealed.

The possibility that cleavage of disulfide bridge occurs when the protein derivatives are solubilized is tested by titrating sulfhydryl groups, using p-chloromercuribenzoate according to the method of Boyer.

Human hairs obtained by epilation are separated into their various components by microdissection. Arginine assays are performed by a microadaption of previous incubation procedures plus the microdiffusion techniques of Conway for determination of the ammonia (resulting from urease hydrolysis of urea).

Evaluation of ornithine transcarbamylase, and arginine synthetase in epidermis is achieved by two methods. The first method utilizes pre-existing techniques, assaying with an excess of substrate in each case, while the second employs radioactive C-14 arginine, and labelled ornithine and citrulline derived thereof, in tracer amounts. Products of the tracer reactions are separated by high voltage electrophoresis, eluted, planchetted and counted in windowless gas flow system.

Major Findings: The peptide patterns obtained for each of the nine different protein fractions reveal major similarities between the protein fractions of each tissue. Although differences are found in the peptide patterns of normal epidermis, psoriasis scale and ichthyosis scale, it is not certain whether these peptide differences are due to abnormal keratin.

Solubilization of protein did not require cleavage of disulfide bonds; however, a difference in hydrogen bonding of the proteins of the epidermal tissue is indicated from the different peptide groups present in the fractions examined.

Preliminary findings indicate that glycine-C^{14} incorporation into the proteins of the epidermis is maximally reflected in the stratum corneum 25-30 days after intravenous administration.

The microdissected components of epilated human scalp hair roots assayed for arginine activity showed activity in the sheaths but not in the bulbar portion of the epilated roots. This finding discounts any local relationship of arginase activity with the protein synthesis of the keratinous material of the hair shaft.

Utilizing the two methods described above, the enzymes of the Krebs-Henseleit urea cycle other than arginase, are found to be absent in fresh surgically obtained human epidermis.

Significance to Cancer Research: Although the diseases discussed in the work are benign, comparison of the protein's synthesized by the cell in normal and hyperplastic epidermis would seem to be important. Evaluation of the differences may also help to indicate the nature of the alteration of normal protein to form abnormal protein.

Other basic information in the process of protein biosynthesis in normal epidermis should aid in evaluation of protein biosynthesis in malignant epidermis.

Proposed Course: Continued evaluation of the "fingerprint" peptide patterns produced when the protein fraction is digest by only one enzyme (trypsin or chymotrypsin), will lead to a clearer peptide pattern and should thus enable a more critical appraisal of the peptide (or amino acids in peptides) differences between the protein fractions.

Studies of the biosynthesis of protein in epidermal tissue in vitro and in humans are now in progress and continuation of these studies is intended.

PHS-398
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Rothberg, S, The Evaluation of Keratin Fractions in Normal and Abnormal Epidermis. J. Invest. Dermat. (in press)

1. General Medicine Branch
2. Metabolism Service
3. Bethesda

FMS-MEM

Individual Project Report
Calendar Year 1959

Part A.

Project Title: Serum Proteins Studies

Principal Investigator: John L. Fahey, M.D.

Other Investigator: For the studies with radio iodinated gamma globulins, Drs. T. Waldmann and Paul Schwab

Cooperating Units: For the study of neoplasms in mice - Laboratory of Biology, NCI (Dr. M. Potter).
For the study of "auto immune" diseases in man - Laboratory of Metabolism, NHI (Dr. E. Goodman).
For the study of red blood cell antibodies - Blood Bank, Clinical Center (Dr. P. Schmidt).

Man Years (calendar year 1959)
Total: 3
Professional: 1
Other: 2

Patient Days (calendar year 1959)

Project Description:

Objectives: The object of this project is to determine physicochemical, metabolic and other characteristics of the serum proteins found normally and in association with malignant disease, the role of selected proteins in the modification or manifestations of disease, and the usefulness of serum proteins as easily accessible indices of disease activity or therapeutic effectiveness.

Methods Employed: Quantitative and qualitative chemical, physical, and immunochemical procedures, as well as tests for biologic activity are used to measure and characterize, where possible, individual proteins. Radioisotopically labelled (I^{131} or C^{14}) proteins in tracer amounts are utilized for metabolic studies. Fractionation of the complex mixture of normal serum proteins are done by electrophoretic, chromatographic, solubility and other procedures.

Patient Material: Sera were obtained from patients admitted to the Metabolism Service, NCI, from cooperating units throughout the National Cancer Institute and the National Institutes of Health and also from physicians in the metropolitan Washington area and throughout the country. Clinical information was available on all of these patients and selected patients were admitted to the Clinical Center for detailed study.

Part B included Yes X No

Major Findings: The gamma globulins of normal serum [illegible] shown to be a large family of related but not identical protein molecules. Subgroups within this protein family were then investigated in detail rather than treating the gamma globulin as a homogeneous unit. Two major groups of gamma globulins, with ultracentrifugal sedimentation coefficients of 19S and 6.6S, can be separated by anion-exchange cellulose chromatography and the latter group can be further subdivided into 4 or more 6.6S fractions. Detailed characterization of these fractions revealed that the 6.6S gamma globulins were, indeed, a family of molecules sharing similar ultracentrifugal and immunochemical properties but differing in electric charge, heme content and antibody activity.

A similar family of molecules was found when myeloma proteins from the sera of 38 patients with multiple myeloma were examined in detail. These myeloma proteins, which were either homogeneous or combinations of a few closely related proteins, appeared to be representative of proteins normally present in serum in small amount. Similar observations were made on the serum myeloma proteins associated with B transplantable mouse plasma-cell neoplasms.

The individuality of the myeloma proteins, coupled with the previous demonstration that myeloma protein is formed in the plasma-cell tumor, indicated that each plasma-cell malignancy was functionally distinct and that each tumor could be identified by its soluble serum product, the myeloma protein.

It follows from the above consideration that, as the variety of myeloma proteins represent a variety of plasma-cell neoplasms, the spectrum of normal 6.6S gamma globulins very likely represent a spectrum of individual normal plasma-cells.

Individuality of gamma-globulin producing cells was also shown in a study of serum antibodies. Many antibodies were found to be 6.6S gamma globulins, but a number of antibodies were found to be 19S gamma macroglobulins. Why an antigen induces a particular type of antibody remains to be determined. The question is further complicated by the observation that different individuals may have different types of antibody to a particular antigen and that several physicochemical types of antibody molecules may be found to a single antigen. Combination of physicochemical and immunological techniques, however, have been developed which permit recognition and investigation of these apparent complexities.

Significance to Human Neoplasia: The evidence presented here is entirely consistent with a unicellular origin of multiple myeloma. Arising from a single cell the neoplasm apparently spreads by metastasis to the multiple sites so frequently encountered in clinical practice.

The myeloma protein has proved to be an important metabolic marker for plasma-cell neoplasms. It is accessible, readily identified and easily quantified. With the demonstration in mouse tumors of a direct correlation between the amount of tumor and the amount of serum myeloma protein, it would seem that serial quantitative measurement of the serum myeloma protein may be more valuable in assessing the clinical course and response to therapy of any individual patient than serial bone marrow aspirations or skeletal roentgenographic examinations particularly in view of the uneven distribution of myeloma cells and the difficulties in evaluating quantitatively the roentgenographic bone changes.

Proposed Course of the Project: It is proposed to investigate the effect of malignant plasma-cells on the remaining normal plasma-cells, the synthesis of myeloma proteins and related proteins by the malignant plasma-cells, the metabolic alterations induced in the gamma globulins by plasma-cell malignancy, and the significance, in terms of normal proteins, of the finding of myeloma proteins of the beta globulin electrophoretic mobility and multiple components, 6.6S - 15S, in the ultracentrifuge, and other properties suggesting a relationship to proteins normally formed in the plasma-cells.

PHS-416
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts:

1. Fahey, J.L. and Horbett, A.P., Fractionation of Gamma Globulin by Anion-Exchange Cellulose Chromatography. J. Biological Chem. 234: 2645-2651, 1959.

2. Fahey, J.L. and Morrison, E.G.
Separation of 6.6S and 18S Gamma Globulins with Isohemagglutinin Activity. J. Laboratory and Clinical Medicine, in press.

3. Goodman, H.C., Fahey, J.L., Malmgren, R.A. and Brecher, G.
Separation of Factors in Lupus Erythematosus Serum Reacting with Components of Cell Nuclei, Lancet, 2: 382-383, 1959.

4. Fahey, J.L. and Potter, M.
Bence Jones Proteinuria Associated with a Transplantable Mouse Plasma-Cell Neoplasm. Nature, 184: 654-655, 1959.

5. Fahey, J.L., Potter, M., Gutter, F.J. and Dunn, T.B.
Distinctive Myeloma Globulins Associated with a New Plasma-Cell Neoplasm of Strain C_3H Mice. Blood, in press.

6. Potter, M. and Fahey, J.L.
Studies on Eight Transplantable Plasma Cell Neoplasms of [illegible]
J. Nat'l. Cancer. Inst., in press.

7. Dunn, T.B., Potter, M., Fahey, J.L. and Merwin, R.L.
Morphology and Serum Protein Changes in Plasma-Cell Neoplasms in Mice. Archivio "del Vecchi" per l'Anatomia Patologica, in press.

1. General Clinical Research
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Studies of Porphyrin Metabolism in the Tumor Bearing Host and Porphyria.

Principal Investigator: Donald P. Tschudy

Other Investigators: Annie Collins, Emmanuel Kellman

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1 3/4
Professional: 3/4
Other: 1

Patient Days (calendar year 1959)

Project Description:

Objectives: To study tumor host interrelationships from the standpoint of porphyrin synthesis in liver and red cells.

To study the effects of vitamins, drugs and hormones on porphyrin biosynthesis.

To study biochemical abnormalities of acute intermittent porphyria.

Methods employed:
A. Quantitative enzyme determinations
B. Assay of urine for porphyrins, porphyrin precursors, uric acid and amino acids
C. Use of C^{14} glucose, acetate and pyruvate in homogenates in measuring their rate of conversions to fatty acids, cholesterol and CO_2.

Patient Material: Red cell enzyme studies will be performed in patients with malignancies and patients with acute prophyria are being studied.

Major Findings: The synthetic analogue of δ-aminolevulinic acid which is a potent inhibitor of ALA dehydrase in vitro has not been active in vivo by methods used thus far.

Attempts to demonstrate a humoral factor produced by tumors which causes the decrease in hepatic ALA dehydrase have been inconclusive. Adrenal cortical hormones diminish the activity of

Part B. included Yes ____ No X

hepatic ALA dehydrase, but the decrease of this enzyme occurs in the adrenalectomized tumor bearing host. The enzyme decrease is therefore not secondary to adrenal cortical activity. Injections of "toxohormone" preparations fail to cause any decrease in ALA dehydrase.

Significance to Cancer Research: The decrease in hepatic ALA dehydrase has been found in all tumor bearing hosts examined thus far (when the tumor is visibly enlarged). This suggests a biochemical abnormality common to malignant tumors. Studies of this abnormality may enable us to understand specific mechanisms whereby a malignant tumor affects the host.

Proposed Course of the Project:

A. To study relationships of carbohydrate metabolism and Krebs cycle activity to porphyrin synthesis and porphyria.

B. To study further chemical abnormalities in acute porphyria.

Serial No. NCI-6-761-(c)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Metabolism of Nutrients and Calories in Neoplastic Disease.

Principal Investigator: Donald M. Watkin, M.D.

Other Investigators: Donald F. Tschudy, M.D., S. I. Berlin, M.D.
Clinical Associates: Peter S. Mueller, M.D., Robert Couch, M.D. and Leon Rosenberg, M.D.

Cooperating Units: Surgery Branch, NCI; Biochemistry Branch, NCI

Man Years: (calendar year 1959) Patient Days (calendar year 1959)
Total: 4.3
Professional: 1.5
Other: 2.8

Project Description:

Objectives: Investigation of the over-all and intermediary metabolism of fat, carbohydrate, protein, minerals, electrolytes, vitamins, calories and water in patients during the natural course of neoplastic disease, and during their responses to nutritional, physiological, pharmacological, endocrine, surgical or radiologic stress.

Methods Employed: 1). The metabolic balance technique to quantify changes in the composition of tumor and host and for measuring the over-all metabolism of nutrients and calories.

2). Calculation of insensible water loss from insensible weight loss and metabolic balance data.

3). Bomb calorimetry for the direct determination of calories in dietary intake, and in urinary and fecal excretions.

4). Measurements of the respiratory quotient (R.Q.), and basal metabolic rate (B.M.R.) by open circuit indirect calorimetry.

Part B included Yes X No ____

5). Estimates of body fat and body density by measurement of skinfold thicknesses by skin-fold thickness calipers and by means of tritiated water* and helium dilution* techniques (* with Dr. H. I. Berlin).

6). Measurement of uric acid in blood, urine, and body tissues and fluids by an enzymatic degradation technique.

7). Measurement of plasma unesterified fatty acids, (U F A).

8). Quantitative extraction of lipids from tissue, food and feces.

9). Protein pool turnover studies using N^{15} labeled amino acids (with Dr. Donald P. Tschudy).

Patient Material:

1)	In-patients, 11-17-58	1
2)	In-patients admitted, 11-17-58 through 11-16-59	
	a) Initial	14
	b) Readmission	5
3)	Normal Controls	22

Major Findings: Interpretations of data from investigations conducted during the past 12 months has advanced appreciably our understanding of a syndrome often observed in patients with neoplastic disease. This syndrome which for purposes of identification will be called the "host-weight loss" syndrome, consists of host weight loss, negative calorie balance, a change in body composition characterized by a decrease in the per cent fat and an increase in the per cent lean body mass, a low basal respiratory quotient (R.Q.) and an increased basal unesterified fatty acid (UFA) concentration.

A. An 11 year old boy with renal aminoaciduria, a unique condition which is analogous to neoplasia in that it robs the body nitrogen pool of amino acids required for synthesis of protein in normal organs and tissues, has demonstrated the key features of the syndrome mentioned above. Supplementation of his daily diet with amounts of threonine (1.0 gm.) and lysine (0.5 gm.) double the quantity being lost in his urine was associated with increased growth, positive N balance and an increase in total daily energy expenditure. N^{15}-labelled aspartic acid turnover studies were performed in this patient to measure nitrogen kinetics at two different levels of protein intake, 4 gm. per kilo of body weight per day and 2 gm. per kilo of body weight per day. Mass spectrographic determinations essential for the interpretation of these studies have not yet been completed.

Significance to Cancer Research: These findings suggest by analogy that neoplastic disease may induce the above mentioned syndrome by extracting amino acids from the body nitrogen pool and that identification of and supplementation of the host dietary with the extracted amino acids may offer a means of reversing the manifestations of the syndrome in the host.

Proposed course of project: Current studies in this patient are testing the hypothesis that the growth observed during threonine and lysine supplementation was the result of the patient's receiving the specific amino acids, not merely nonspecific nitrogen. Projected studies include the formulation from urinary amino acid excretion data quantified by column chromatography of a chemically defined dietary supplement which will provide this patient with a total dietary of the highest biological value. This supplement will then be tested for its ability to reverse the manifestations of the host-weight-loss syndrome which this patient displays. During these investigations the relationship of total caloric expenditure to protein synthesis will be quantified by the measurement of insensible water loss, by indirect calorimetry and by changes in body composition between two points in time. N^{15}-labelled aspartic acid turnover studies will be incorporated into the experimental design to provide information on nitrogen kinetics for comparison with the two previous studies performed at two levels of protein intake which were mentioned above.

B. Previous investigations of this project have demonstrated that ingestion of normally adequate dietary protein and caloric hyperalimentation with intravenously administered fat emulsions stimulate tumor protein synthesis more than host protein synthesis. Other studies before, during and after chemotherapy of myeloproliferative diseases have shown that, only after reduction of tumor protein synthetic capacities is the host able to respond to the protein anabolic effects of diet. These findings suggested that a potent physiologic anabolic stimulus for the host which would not at the same time either stimulate or poison the tumor would permit measurement under truly physiologic conditions of host-tumor competition, particularly for amino acids and calories, under circumstances such that the host would have more of a competitive advantage than in more conventional experimental designs. Exercise was selected as the most physiologic anabolic stimulus known.

Five young patients with neoplastic disease have been observed before, during and after many consecutive days of exercise on a motor-driven treadmill. Four of these whose studies have been completed (one is still in progress) showed the following in association with exercise: 1) loss of P possibly due to loss of tissue high in P and low in N and reutilization of N; 2) a decreased excretion of uric acid; 3) a lowered basal respiratory quotient; 4) a lowering of increased UFA concentrations due to active neoplastic disease but an increase in UFA concentrations already in the normal range; and 5) a change in body composition characterized by a loss of fat and water.

Significance to Cancer Research: These studies suggest that organ and tissue anabolism can occur under the influence of a normal physiologic stimulus in the presence of neoplastic disease. The changes in the parameters measured may be interpreted as showing that exercise does stimulate effective host competition with tumor for the ingredients of protein synthesis.

Proposed course of project: Completion of present study of a patient with chronic myelocytic leukemia, an ideal disease for study because of its long and benign course and the multiple, easily measurable parameters it affords for study. Recruitment and study of suitable normal controls in the same age group. Determination of the quantitative relationships between total body caloric expenditure and the synthesis of protein during exercise in tumor-bearing and nontumor-bearing hosts.

C. Previous investigations under this project have demonstrated that the basal respiratory quotient ($R.Q._o$) is low, the basal plasma unesterified fatty acid (UFA) concentration high and the basal metabolic rate (BMR) variable from very low to very high in patients with active neoplastic disease who are consuming an adequate dietary.

To examine the metabolic rate (MR), the $R.Q._o$ and the plasma UFA concentration throughout the day as well as under basal conditions, 16 "feeding-fasting" studies were performed in 13 patients. During the first day, the patient ate his usual constant diet. From the evening of the first day to the morning of the next following day, he fasted. Measurements of the metabolic rate, the $R.Q._o$, and the plasma UFA concentrations were made periodically throughout the study.

Patients with active neoplastic disease either showed a low $R.Q._o$ and high UFA at the beginning of their fast period or demonstrated a change to low $R.Q._o$ and high UFA within 12 hours of the beginning of the fast. Patients with inactive disease and normal subjects required from 16 to 24 hours and sometimes 36 hours to reach the same low $R.Q._o$'s and high UFA concentrations observed in the patients with active neoplastic disease. The metabolic rate remained elevated in patients with extremely active disease despite fasting, whereas in patients with less active disease and in normal subjects the metabolic rate was found to be lower at any particular point in time relative to the "feeding" day.

Significance to Cancer Research: These studies demonstrate that patients with active neoplastic disease respond to fasting by a shift from a metabolic mixture with an $R.Q._o$ of 0.8 to 0.85 to a metabolic mixture with an $R.Q._o$ of 0.65 to 0.7 and by increasing their plasma UFA concentrations as a means of mobilizing fat in far shorter times than do patients with inactive disease or normal subjects. There is no direct relation between metabolic rate and changes in $R.Q._o$ and UFA concentrations, since several patients with active [illegible] had normal or even low metabolic rates. These

findings suggest the presence of an abnormality in lipid mobilization and utilization in the patient with active neoplastic disease not noted in normal subjects or in patients ill with other diseases.

Future course of project: Additional studies in patients with very active neoplastic disease and in other nonneoplastic conditions associated with hypermetabolism such as pregnancy, hyperthyroidism, fever, acute anxiety and chronic exercise.

B. As a step in developing further the role of the amino acids in the genesis of the host-weight-loss syndrome, the efficacy of water soluble chemically defined diets (supplied by the Biochemistry Branch) in host maintenance and repletion were studied in two patients transferred to the Metabolism Service from the Surgery Branch with inactive neoplastic disease.

Data from these studies indicate that diets containing L-amino acids are suitable for both maintenance and repletion but are more efficacious when given at the 50 calorie per kilo of body weight level than at the 35 calorie per kilo of body weight level. They also suggest that D-L amino acid mixtures may also be efficaciously used by the host after an equilibration or adjustment period of about 6 days.

Significance to Cancer Research: These diets may be varied in their composition exactly to conform to the optimum biological value for a given patient. Hence they provide an invaluable tool with which to approach the difficult problem of the role of the amino acids in the genesis of the host-weight-loss syndrome.

Future course of project: Investigation into the reason behind the observation that purified amino acid diets require more calories for a given degree of nitrogen retention than do isonitrogenous diets composed of whole protein. As greater supplies of amino acids are available, studies to develop specific mixtures of amino acids with highest biological values for normal subjects and for patients with one specific neoplastic process will be undertaken.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1) Levenson, S.M., and Watkin, D.M., Protein requirements in injury and certain acute and chronic diseases. Federation Proceedings, December, 1959 (In Press). pp. 31-66.

2) Gyorgy, P., Chairman; Wallace, W.M.; Hegsted, D.M.; Harper, A.E.; Waterlow, J.C.; Watkin, D.M.; Ross, M.H.; and Scrimshaw, N.S. Symposium on protein requirement and its assessment in man. Federation Proceedings, December, 1959 (In Press).

3) Shaw, R.K., Moore, E.W., Mueller, P.S., Frei, E., III, and Watkin, D.M. The effects of actinomycin D on childhood neoplasms. A.M.A. J. Dis. Child. (In Press).

4) Watkin, D.M. Protein nutrition in neoplastic disease, Food and Nutrition News, 30: issue #9, pages1 & 4, June 1959.

The following manuscript has been submitted for approval: [illegible] and Watkin, D.M. Plasma unesterified fatty acid (UFA) concentration[illegible] neoplastic disease. (For publication in J. Clin. Invest.)

As of November 15, the following manuscript is in the process of final typing prior to submission for approval: Watkin, D.M., and Steinfeld, J.L. Metabolic relationships between tumor and host during large-dosage prednisone therapy in human neoplastic disease. (For publication inJ.N.C.I.)

Serial No. NCI-6-723(c)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Studies of Human Serum Albumin Labelled with I^{131}

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Robert Gordon, M.D. (NHI) and Julian White, Ph.D.

Cooperating Units: Laboratory of Physiology National Cancer Institute
National Heart Institute

Man Years (calendar year 1959)
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1959)

This project was divided into Projects I and II. See attached.

Serial No. NCI-6-723(c)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959
Project I

Part A:

Project Title: Distribution and Degradation of Human Serum Albumin Labelled with I^{131}.

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Julius White, Ph.D.

Cooperating Units: Laboratory of Physiology, National Cancer Institute.

Project Description:

Objectives: To determine the controlling mechanisms of albumin synthesis and degradation in normals and patients with cancer.

Methods Employed: I^{131} albumin is given intravenously to individuals receiving Lugol's Solution. Blood and 24 hour urine and stool collections are assayed for radioactivity for 28 days to determine the albumin degradation kinetics. N^{15} Glycine is given intravenously, and its uptake into serum albumin and globulin is determined by mass spectrometry.

Patient Material: The studies were performed on a patient with a cerebellar hemangioblastoma.

Major Findings: The percent of the body albumin degraded per day was increased 60 percent and the rate of albumin synthesis decreased approximately 40 percent following daily infusion of 50 grams of albumin a day for 10 days.

Significance to Cancer Research: This study is directed toward determining the factors involved in the control of the production and destruction of serum albumin, as a part of a study to determine the cause of the hypoalbuminemia in cancer patients.

Proposed Course of Study: To continue the studies to determine the effect of alterations of the albumin and gamma globulin pool sizes on the rate of synthesis of these proteins.

Part B included: Yes___ No X

Serial No. NCI-6-723(c)

1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959
Project II

Part A:

Project Title: The Distribution and Degradation of I^{131} Albumin and I^{131} Polyvinylpyrollidone in Patients with Idiopathic Hypoproteinemia and Gastrointestinal Neoplasms

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Robert S. Gordon, M.D.

Cooperating Units: National Heart Institute, LCPM.

Project Description:

Objectives: To study the pathogenesis, clinical pattern and the kinetics of albumin destruction in patients with idiopathic hypoalbuminemia.

Methods Employed: I^{131} labeled albumin is given intravenously to an individual receiving Lugols solution. Blood and 24 hour urine and stool samples are assayed for radioactivity. I^{131} polyvinylpyrollidone (PVP) is given intravenously and stools collected in the subsequent 4 days are assayed for radioactivity.

Patient Material: 30 I^{131} albumin studies have been performed in 18 patients with idiopathic hypoalbuminemia.

Major Findings: On the basis of the I^{131} albumin studies and clinical data patients with idiopathic hypoalbuminemia fall into two major groups the hypoanabolic and hypercatabolic. The hypoanabolic patients have an isolated deficit in albumin synthesis with prolonged I^{131} albumin survival and secondary hyperglobulinemia and hypercholesterolemia. All of these abnormalities are corrected by albumin infusion. The hypercatabolic patients have normal or slightly increased albumin synthesis but rapid degradation or loss of albumin. All of the patients with idiopathic hypercatabolic hypoproteinemia and with ulcerative gastrointestinal diseases show an elevation of the macromolecule I^{131} PVP in the 4 day stool collection following intravenous injection compared to normals and patients with diseases not affecting the

Part B included Yes X No

gastrointestinal tract. This suggests that the gastrointestinal tract is the site of protein loss in patients with idiopathic hypercatabolic hypoproteinemia.

Approximately 25 percent of the patients with this syndrome reported in the literature have had idiopathic chylous effusions. At laparatomy or Crosby capsule biopsy 5 of 6 patients studied have shown markedly dilated lymphatic channels in the jejunal submucosa.

Relationship to Cancer Research: Loss of protein into the gastrointestinal tract may be factor in the hypoalbuminemia associated with gastrointestinal cancer.

Proposed Course: To continue the studies directed toward understanding the pathogenesis of hypoalbuminemia both idiopathic and that associated with neoplastic diseases.

PHS-NIH
Individual Project Report
Calendar Year 1959
Project II

Part B: Honors, Awards, and Publications

Publications other than abstracts:

1. Gordon, R.S. Jr., Bartter, F.C., Waldmann, T.A. Idiopathic Hypoalbuminemia: Clinical Staff Conference of the National Institutes of Health. Annals of Int. Med. 51: 553-576, 1959.

Serial No. NCI-6-725-(c)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-410
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Vitamin B_{12} metabolism in normal subjects and patients with neoplastic and other diseases.

Principal Investigator: Donald M. Watkin, M.D.

Other Investigators: I. Bernard Weinstein, M.D.

Cooperating Units: None

Man Years (calendar year 1959)
Total: 2 1/3
Professional: 1 1/3
Other: 1

Patient Days (calendar year 1959)

Project Description:

Objectives: Quantitative observations on the metabolism of vitamin B_{12} in man including its absorption from the gastrointestinal tract, its distribution in the organs of the body, its level in plasma, its binding to plasma proteins and its excretion by the kidney in normal subjects and those with neoplastic disease.

Methods Employed: Microbiological assay (*L. leichmannii*) for vitamin B_{12} in plasma, urine and tissue. Measurement of Co^{58}-labelled vitamin B_{12} in blood, plasma, plasma protein fractions, urine, feces and tissues *in vitro*, and in organs *in situ* by *in vivo* scanning. Paper and block electrophoresis and column chromatography of plasma proteins. Dialysis and ultrafiltration of plasma proteins. Renal clearance techniques. Perchlorate precipitation procedure. Studies of B_{12} antagonists and analogs in bacterial, tissue culture, plasma and mammalian systems.

Patient Material: Fourteen admissions on 11 patients. Of these 6 had chronic myelocytic leukemia, 2 had Addisonian pernicious anemia, and 1 each had myeloid metaplasia, acute myelocytic leukemia, carcinoma of the pancreas, and carcinoma of the lung.

Major Findings: The B_{12}-binding protein of the plasma of normal subjects and of subjects with chronic myelocytic leukemia has been found in the seromucoid fraction of plasma and displays the characteristics of a glycoprotein. Further purification of this seromucoid fraction has been

Part B included Yes X No

achieved by anion-exchange cellulose column chromatography. The seromucoid fraction of normal plasma appears to have three components to which B_{12} is bound. In the seromucoid fraction of plasma from patients with chronic myelocytic leukemia, there is a marked increase in a B_{12}-binding component whose elution characteristics are the same as one of the components in normal plasma. Attempts to establish that the B_{12}-binding component of plasma can substitute for intrinsic factor in the gastrointestinal absorption of B_{12} have been inconclusive in two patients with Addisonian pernicious anemia.

A series of studies with the methylamide and enalide analogs of a monocarboxylic form of cyanocobalamin (obtained from Dr. E. Lester Smith of the Glaxo Laboratories) were carried out in vitro with E. histolytica and in vivo using Co^{58}-labelled vitamin B_{12} which indicated that these compounds could compete effectively with cyanocobalamin for binding sites on plasma and in gastrointestinal absorption on intrinsic factor. However, clinical studies in 2 patients with chronic myelocytic leukemia and high plasma B_{12} concentrations controlled by parallel studies in 2 patients with adenocarcinoma and normal plasma vitamin B_{12} concentrations failed to reveal any therapeutic effect on chronic myelocytic leukemia.

Significance to Cancer Research: The results of these studies suggest that the abnormally high plasma B_{12} concentrations in myeloproliferative disorders are caused by the presence of an increased quantity of a normally occurring component of the seromucoid fraction of plasma. These results therefore are clear indications for additional work on the kinetics of this component and on the precise role of the increased quantity of B_{12} in plasma in the myeloproliferative process. Studies with the B_{12} antagonists indicate that these compounds have certain properties of B_{12} analogs but that these are not clinically important. They do however provide encouragement for further efforts on the part of the vitamin chemists to produce similar analogs in the hope that some may prove clinically effective.

Proposed course: Studies now in progress designed to estimate the plasma clearance and the urinary, fecal and biliary excretion of Co^{58}-labelled vitamin B_{12} administered intravenously bound to the patient's own plasma protein will be carried out in patients with chronic myelocytic leukemia before and after therapy and in other patients with and without neoplastic disease.

Concentration of the active component of the seromucoid fraction of plasma from patients with chronic myelocytic leukemia and its further subfractionation and purification will be attempted. Additional studies will also be performed to demonstrate conclusively whether or not this fraction will substitute for intrinsic factor in the patient with Addisonian pernicious anemia.

Recently an ethylamide derivative of the monocarboxylic form of

cyanocobalamin has been obtained from Dr. E. Lester Smith of the Glaxo Laboratories. This derivative should be investigated in the same manner used in examining the methylamide and the analide derivatives. Studies designed to explain the paradox of the fact that these compounds compete effectively for binding sites on plasma and intrinsic factor and yet appear to be biologically inactive in mammalian systems will be carried out.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1) Weinstein, I.B., Weissman, S.M., and Watkin, D.M. The plasma vitamin B_{12} binding substance: I. Its detection in the seromucoid fraction of plasma from normal subjects and patients with chronic myelocytic leukemia. J. Clin. Invest. 38: 1904-1914, 1959.

The following manuscripts from this project have been submitted for approval:

Weinstein, I.B., and Watkin, D.M. $Co^{58}B_{12}$ absorption, plasma transport, organ distribution and excretion in patients with myeloproliferative disorders, solid tumors and nonneoplastic disease. (To be submitted for publication to J. Clin. Invest.)

Weinstein, I. B., Weissman, S.M., and Watkin, D.M. The plasma vitamin B_{12} binding substance: II. Further purification from normal and chronic myelocytic leukemia plasma by fractionation of seromucoid on anion-exchange cellulose columns. (To be submitted for publication to J. Clin. Invest.)

Serial No. NCI-[illegible]
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

Individual Project Report
Calendar Year 1959

Part A:

Project Title: Renal clearance studies in patients with neoplastic disease.

Principal Investigator: Donald M. Watkin, M.D.

Other Investigators: I. Bernard Weinstein, M.D.

Cooperating Units: None

Man Years (calendar year 1959) Patient days (calendar year 1959)
Total: 1/14
Professional: 1/48
Other: 1/48

Project Description:

Objectives: To determine the glomerular filtration rate, effective renal plasma flow and tubular mass and to investigate the mechanisms by which the kidneys handle metabolites such as uric acid and calcium in patients with neoplastic disease. To ascertain the kidneys' role in such disorders as hyperuricemia and hypercalcemia when these accompany neoplastic disease.

Methods Employed: Renal clearance technique. Enzymatic assay for uric acid. Ultrafiltration procedure for estimating filterable [illegible]

Patient Material: Two renal function studies were performed, one in each of two patients with cryoglobulinemia.

Major Findings: Urate reabsorption is a linear function of the filtered urate load. In individuals at the extremes of filtered load, reabsorption deviates from the linear function. Correction of hypercalcemia results in a marked increase in GFR, ERPF and TmUA. Hypercalcemia due to a diminished reabsorption of urate may be a characteristic of patients with [illegible]. One patient with cryoglobulinemia reabsorbed only 60% of filtered urate load. However, the other patient reabsorbed the normal 90% of filtered load.

Significance to Cancer Research: Plasma uric acid level is [illegible] in part by renal tubular reabsorption of urate, in part by uric acid synthesis, and in part by the release of uric acid and its precursors

Part B included Yes____ No X

from tissues undergoing destruction. The renal contribution to the maintenance of plasma level must be known before hyperuricemia may be ascribed to increased synthesis or to tumor destruction. Hypercalcemia is a dangerous complication of neoplastic disease. As in the case of hyperuricemia, investigations of the renal mechanisms for clearing calcium in hypercalcemia are basic to an understanding of the syndrome.

Proposed Course:

1) Additional clearance studies in patients with hyperuricemia, especially those with plasma levels sufficiently high to result in filtered urate loads exceeding Tm-urate.

2) Collect clinical laboratory data on such procedures as concentration tests, blood urea nitrogen levels, creatinine levels, microscopic examination of urine sediment, examination of urine for proteinuria, PSP excretions, creatinine clearances and intravenous pyelograms for comparison with discreet renal function studies so that the reliability of clinical indices of renal function in neoplastic disease may be evaluated.

3) Correlate data obtained by clearance studies with histology of the kidney as seen in renal biopsies and autopsy material.

4) Develop a reliable technique for determining filterable calcium in plasma and test its reliability and reproducibility.

5) Clearance studies in patients with hypercalcemia to determine the factors influencing tubular reabsorption of calcium and its relation to tubular handling of urate and phosphorus.

6) Further studies of protein fraction clearances in selected patients with proteinuria.

7) Study additional patients with aminoaciduria.

Serial No. NCI-G-727(c)
1. General Medicine Branch
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Gross Body Composition

Principal Investigator: N. I. Berlin, M.D.

Other Investigators: D. M. Watkin, M.D.

Cooperating Units: None

Man Years (calendar year 1959) Patient Days (calendar year 1959)
Total: 4
Professional: 4
Other: 0

Project Description:

Objectives: This project is designed to study the changes in gross body composition during the course of neoplastic diseases, and to correlate these changes with the clinical course of the patient and other laboratory data, and to study the anemia of cancer in the same group of patients.

Methods Employed: Gross body composition will be determined from the body density and total body water. Body density will be determined from body mass and body volume, as measured by the Siri body volume apparatus; body water will be determined with tritiated water. Hematological status will be determined by measurement of total red cell volume with Cr^{51}, iron turnover with Fe^{59}, and red cell life span with C^{14}.

Patient Material: 6 patients with various neoplasms; 4 subjects (with Drs. Rhoden and Bushkirk, NIAMD); 6 patients (4 with neoplasms, not studied on a metabolic balance regime)

Major Findings:

1. Gross Body Composition:

Modification of the method of analysis of helium and electronic measurement and recording of the temperature in the chamber have been satisfactorily completed. In general, replicate runs on a patient do not differ more than 100 cc.

Analysis of the changes in body composition in 3 obese subjects has been completed. Excellent agreement between the calculated fat loss as measured in this project and from metabolic balance data was obtained. The combination of these 2 procedures furnishes an adequate and satisfactory approach to the study of both short and long term changes in gross body composition.

Part B Included Yes X No

It is estimated that the standard deviation in the difference between 2 estimates of total body fat, from densitometry and body water measurements is approximately 0.5 kg.

Analyses of the other patients studied awaits the completion of metabolic balance data.

2. Anemia of cancer:

Two patients with myeloid metaplasia have been treated with large doses of testosterone. One patient died of massive bleeding before a therapeutic effect could be achieved. The second patient shows objective evidence of a hematological response; as measured by body density and water this patient showed an increase in body fat while under therapy; the metabolic balance data are not yet available.

Significance to Cancer Research: This project is part of the studies carried out on the area of the tumor-host relationship. During the course of a neoplastic disease, large changes in body weight occur, particularly weight loss. These must represent some changes brought about in the metabolism of the host. As a preliminary and a necessary adjunct to the study of these changes in the host's metabolism, it is necessary to know the changes occurring in gross body composition.

Anemia occurs in cancer to a variable degree depending upon the criterion of anemia selected. Presently available methods permit the quantitation of erythropoiesis. Correlation of the level of erythropoiesis with changes in body composition will afford an opportunity to compare the metabolism of a single system with that of the whole body.

Proposed Course: The initial phase of installation and modification of the equipment has been completed. The data from the 3 obese subjects indicate that the goals originally set can be met. At present, recruitment and long term follow-up of selected patients is underway.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts:

1. Nathan, D.G., Davidson, J.D., Waggoner, J.G., and Berlin, N.I. The Counting of Barium Carbonate in a Liquid Scintillation Spectrometer. J. Lab. and Clin. Med. 52: No. 6, 915-917, 1958.

2. Nathan, D.G., Berlin, N.I. Studies of the Production and Life Span of Erythrocytes in Myeloid Metaplasia. Blood. 14:No. 6, 668-682, 1959.

3. Nathan, D.G. and Berlin, N.I. Studies of the Rate of Production and Life Span of Erythrocytes in Acute Leukemia. Blood 14: No. 8 935-949, 1959.

1. General Medicine B
2. Metabolism Service
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Studies of Nitrogen Metabolism using N^{15}.

Principal Investigator: Donald P. Tschudy

Other Investigators: Emmanuel Hellman

Cooperating Units: Laboratory of Physiology

Man Years (calendar year 1959)
Total: 2 1/4
Professional: 1 1/4
Other: 1

Patient Days (calendar year 1959)

Project Description:

Objectives:
A. Through the use of mathematical models of nitrogen metabolism to study the effects of such variables as diet, hormones, tumor growth and radiation on the kinetics of body nitrogen.

Methods Employed:
A. Synthesis of N^{15} labelled amino acids
B. Isolation of urea and ammonia from urine
C. Preparation of materials for mass spectrometry
D. Metabolic balance studies

Patient Material: A patient with lymphosarcoma has been studied through most of the course of her disease. Patients with malignancies and metabolic abnormalities will be studied.

Major Findings: The growth of a local tumor mass (lymphosarcoma) in the leg caused a slight increase in body nitrogenous metabolic pool size with no change in rate constant for metabolic pool turnover. There was a slight increase in daily body nitrogen incorporation. Irradiation of the leg mass (1000 r - 1 dose) caused marked enlargement of body nitrogenous metabolic pool with great decrease in rate constant for metabolic pool turnover. Body nitrogen incorporation remained essentially unchanged.

Part B included Yes X No

Significance to Cancer Research: These methods permit the quantitative study of the tumor bearing host with regard to nitrogen metabolism and the effects of many variables such as diet, therapy, etc. thereon.

Proposed Course of the Project:

A. To study the effects of radiation, steroids and tumor growth more extensively.

Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Studies of the Effect of Dietary Protein and Caloric Levels on the Kinetics of Nitrogen Metabolism Using N^{15} L-Aspartic Acid. Donald P. Tschudy, Habeeb Bacchus, Sherman Weissman, Donald M. Watkin, Marjorie Eubanks and Julius White, J. Clin. Investigation 38, 892-901, (1959)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Investigation of Malignant Reticular Disease and [illegible] Protein Abnormalities.

Principal Investigators: John L. Fahey, M.D.

Other Investigators: None

Cooperating Units: In specific aspects of the work,
Pathologic Anatomy Branch, NCI
(Drs. T. Dutcher and R. MacCardle).
Laboratory of Biology, NCI
(Dr. J. Dalton)
Institute for Allergy and Infectious Diseases
(Dr. J. Utz)

Man Years (calendar year 1959)
Total: 1 1/2
Professional: 1
Other: 1/2

Patient Days (calendar year 1959)

Project Description:

Objectives: The clinical and biochemical characteristics of malignant reticular and related diseases are to be defined and explored with particular attention to macroglobulinemia, cryoglobulinemia or other significant serum protein abnormalities. It is hoped thus to gain insight into the mechanisms responsible for the major disabling features of these illnesses, to clarify diagnosis and prognosis, and to improve treatment.

Methods Employed: Chemical and physicochemical techniques, including electrophoresis, ultracentrifugation and chromatography are employed for quantitative and qualitative assessment of serum protein changes. Quantitative immunologic techniques are employed for assessment of serum antibody levels and of response to antigenic stimulation. Histologic evaluation is conducted by histochemical immunohistologic, electron microscopic and standard procedures. Rigorous clinical evaluation according to a specific protocol based on previous experience.

Part B included Yes X No___

Major Findings: Gamma macroglobulinemia may occur in patients with a variety of clinical and laboratory manifestations. Morphologic studies (Butcher and Fahey) demonstrated the presence of characteristic lymphocytoid plasma-cells with intranuclear inclusions in patients with malignant disease and macroglobulinemia. The intranuclear (and intracytoplasmic) inclusions were shown to be remarkably similar to the circulating gamma macroglobulin suggesting a common identity and indeed that the lymphocytoid plasma-cells are the site of macroglobulin formation.

Blood viscosity may be markedly elevated by large amounts of serum macroglobulins. Direct demonstration that macroglobulin induced elevated blood viscosity was the immediate cause of cardiac failure in one patient with limited but otherwise adequate cardiac reserve permitted establishment of a therapeutic regimen designed to keep the serum macroglobulins below the critical level.

Significance to Cancer Research: The finding of a characteristic cell in macroglobulinemic lymphoma should improve the recognition of this disease. It will be of particular interest to know if related normal cells are responsible for gamma macroglobulin synthesis, for the gamma macroglobulins normally contain a special group of antibodies as noted in the NCI-6-732(c). The cells of formation for these antibodies are important to immunologists and oncologists concerned with tissue transplantation and immunity.

Recognition of the symptoms of excessive blood viscosity will allow prompt therapy to avert what may otherwise be a progressively deteriorating clinical course.

Proposed Course of the Project: Therapeutic observations in macroglobulinemic diseases will continue with similar observations in cryoglobulinemic diseases. The effects of malignant and non-malignant diseases with dysgammaglobulinemias on normal gamma globulin components and immune mechanisms will be explored. An effort will be made to define the criteria by which non malignant diseases with associated cryoglobulins or increased gamma macroglobulins may be distinguished from malignant diseases with similar changes.

Part E: Honors, Awards, and Publications

Publications other than abstracts:

1. Batchelor, T.P. and Fahey, J.L.
The H chopathology of the Macroglobulinemia of Waldenström
J. Nat. Cancer Inst. 22: 807-817, 1959.

2. Zahred, G.G., Milbish, T.F., Fahey, J.L., Batcher, T.E., Fox, J.J. and Osserman, E.F.
Waldenström's Macroglobulinemia, Annals of Int. Med. 50: 1010-1027, 1959.

... Rehabilitation Service
S. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: A Study of the Factors Controlling Red Blood Cell Synthesis and Destruction.

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Nathaniel I. Berlin, M.D., Sherman M. Weissman, M.D. and Edgar Levin, M.D.

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1959)

Project Description:

Objectives: To study the factors controlling red blood cell synthesis and destruction in normal and in pathological states.

Methods Employed: Quantitation of erythropoiesis by the use of Cr^{51} labeled red cells to determine the total circulating red cell volume, Fe^{59} labeled siderophilin to determine the rate of red cell production and C^{14} labeled glycine to determine the red cell life span. Assay of erythropoiesis stimulating activity in the fasted rat by the red cell radio-iron uptake and reticulocytosis.

Patient Material: A patient with a cystic cerebellar hemangioblastoma and polycythemia.

Major Findings: The studies of splenectomy and methylcellulose infusion in the dog, in part previously reported, have been completed. Following splenectomy in the dog, there was a 31 percent decline in the total circulating red cell volume persisting for at least the remaining 5 months of the study. No change was noted in the red cell life span but there was a 36 percent decrease in red cell synthesis as estimated by the iron red cell turnover. The reticulocyte percentage increased 138 percent as a result of an increased intravascular reticulocyte maturation time following splenectomy. No significant hematological changes were noted in sham operated controls.

Part B included Yes x No

We have utilized methylcellulose infusions in the dog in an attempt to produce an experimental model of hypersplenism. Although moderate splenomegaly and anemia with a shortened red cell survival was achieved the animals developed marked renal damage and uremia. Following administration of 125 grains of desiccated thyroid daily to dogs there was a 25 percent increase in the total red cell volume. There was a comparable increase in the rate of red cell synthesis but no change in the red cell life span.

Arterial shunt studies directed toward determining the site of erythropoietine production have been continued but no new conclusions are available at this time.

A non-dialyzable factor with marked erythropoiesis stimulating activity was isolated from a cystic cerebellar hemangioblastoma in a patient with the association of this tumor and polycythemia. This is the first demonstration of erythropoietine like activity in any material other than urine or plasma. It supports the concept that the polycythemia associated with some cerebellar hemangioblastomas is caused by the production of a red cell stimulating factor by the tumor.

Significance to Cancer Research: Anemia is a major complication of the neoplastic diseases. The pathogeneses of the anemia is only partially understood. A knowledge of the factors involved in erythropoiesis both in normal and decreased states is required.

The studies performed on the patient with cerebellar hemangioblastoma and polycythemia have aided our understanding of the pathogenesis of this association.

Proposed course of Study: To continue the study of the factors controlling erythropoiesis in the experimental animal and man. To conduct studies directed toward determining the chemical nature and mode of action of the cerebellar erythropoiesis stimulating factor.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts:

1. Berlin, N.I., Waldmann, T.A., Weissman, S.M.
Life Span of Red Blood Cell. Physiol. Rev. 39:
577-616, 1959.

2. Weissman, S. M., Waldmann, T.A., Berlin, N.I.
Quantitative Measurement of Erythropoiesis in the
Dog. Am. J. Physiol. in press.

3. Waldmann, T.A., Weissman, S.M., Berlin, N.I.
The Effect of Splenectomy on Erythropoiesis in
the Dog. Blood, in press.

Serial No. NCI 852
1. Pathologic Anatomy Branch
2. Cytodiagnostic Service
3. Bethesda 14, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A.

Project Title: Exfoliative Cytology Applied to Human Diagnostic Problems and Research Problems

Principal Investigator: Dr. Richard A. Malmgren

Other Investigators: Dr. Elizabeth W. Chu

Cooperating Units: None

Man Years (Calendar year 1959):
Total: 8
Professional: 4
Other: 4

Project Description:

The primary responsibility of the Cytodiagnostic Service is to provide the staff of the Clinical Center with an accurate and complete exfoliative cytology service. In addition, the Cytodiagnostic Service collaborates in various clinical research projects evaluating cancer therapy, the hormonal status of the cancer patient, the course and natural history of the cancer lesion, and the anatomical and physiological changes in the human body associated with various pathologic conditions. The accomplishment of these objectives necessitates the continual improvement in techniques and the origination of new cytologic methods in the laboratory.

Methods Employed:

The standard Papanicolaou staining procedure is applied to preparations of various human specimens. These specimens are then screened and evaluated for morphologic changes in the cellular elements. Special procedures involving the use of fluorescent microscopy, millipore filtration, and various special staining techniques are required in many instances.

The figures compiled below are for the last twelve months (12).

Type of material examined	Accessions	# Slides
Wound washings	126	685
Bronchial washings and sputa	116	324
Vaginal and cervical	1,079	2,961
Miscellaneous Fluids (gastric, prostatic, breast, pleural, etc.)	531	1,388
Improvement techniques	186	439
Bloods	1,509	5,961
TOTAL	3,547	11,758

Institute	Accessions	# Slides
NCI	2,450	9,076
NHI	140	414
NIAMD	182	658
NIAID	188	417
NINDB	122	369
NIMH	3	8
NIDR	10	50
Referred	451	761

Major Findings:

1. The major contribution of the Cytodiagnostic Service to the diagnostic facilities of the clinical staff has been the early diagnosis of neoplasms of the cervix and lung and of other sites not previously diagnosed. In several instances, exfoliative cytology provided the only tissue confirmation of neoplastic disease before death of the patient.

2. The quantitative evaluation of wound washings, submitted by the Surgery Branch, NCI has been continued with the demonstration of neoplastic cells in many of the washings.

3. In collaboration with the Surgery Branch, NCI and the U. S. Public Health Service Hospital, Baltimore, approximately 1500 blood specimens will have been collected this year from cancer patients for examination for cancer cells. Cytologic examination of this material by the streptolysin O millipore technique indicates that cancer cells are more often found in cases of non-resectable cancer than in resectable cases. The cells are more frequently found in local blood from the tumor site than from peripheral blood. In a number of patients in which cancer cells have been found in the local blood there has been no recurrence of the disease to date following surgical treatment.

4. The use of the sex chromatin determination on patients with various endocrine disorders has contributed to the ultimate clinical management of these patients.

Major Findings (Continued):

5. The application of the millipore filtration technique to various extra vaginal cytology specimens has increased the efficiency and decreased the time required to process the specimen. It has also permitted a diagnosis to be made which would have been less likely by the older processing methods.

6. The fluorescent antibody technique for the demonstration of antinuclear substances in the serum has been applied to the diagnostic study of systemic Lupus erythematosis and related diseases. The development of this procedure was done in collaboration with Dr. Howard Goodman of the National Heart Institute and the method is now employed in the routine study of patients with Lupus, rheumatoid arthritis, Sjogren's disease and other collagen diseases.

7. Examination of approximately 70 urine specimens using the millipore filter technique in collaboration with Dr. George Owen of NIAID has revealed the presence of intracytoplasmic bodies in macrophage like cells. These bodies were observed in the cells of virtually all the specimens examined regardless of the age, sex or medical status of the patient. This observation is in disagreement with the report of others who found intracytoplasmic bodies of the same sort in association with viral diseases, particularly measles.

Significance of the Program:

1. The diagnostic value of exfoliative cytology in the clinical evaluation of cancer and its early detection is readily apparent from the activities of the Cytodiagnostic Service in the Clinical Center and throughout the country.

2. The wound washing program contributes to a better understanding of the factors involved in tumor growth, progression and metastases and the reaction of the individual to his neoplasm. In addition, the application of this information to cancer chemotherapy may be of value.

3. The fluorescent antibody technique applied to serum of patients with arthritis, systemic Lupus erythematosis and collagen diseases provides one of the most sensitive tests available for the demonstration of antinuclear bodies in the serum. Its use contributes to the complete evaluation of patients with these diseases and may be of help in gaining a better understanding of the etiology of these diseases.

4. By the use of sex chromatin counts and techniques for hormonal evaluation the endocrinologist is provided with a valuable tool for guidance in diagnosis and therapy.

5. The study of tumor cells in the blood presents the potential of gaining a better understanding of the factors involved in metastasis. It may be of value in studying certain types of therapy of the neoplastic diseases and diagnosis.

Significance of the Program (Continued):

6. The development of new cytologic techniques and the acquisition of specimens from different sites in various diseases continues to enhance the value of the exfoliative cytology procedure as a diagnostic aid. The value of the millipore filter technique is readily apparent and it is anticipated that this procedure will replace the smear preparations in many cytologic laboratories.

7. Excretion of active virus in the urine is a well documented phenomenon in some human viral diseases. Whether the virus is intracellular or extracellular at this time is not clear however. The development of methods for the demonstration of virus in the urine is therefore of potential value in the armamentarium of virus study and in the expansion of our knowledge of viral diseases.

Proposed Course of the Project:

1. The continuation of a complete and adequate cytologic service for the staff of the Clinical Center is our primary purpose. Continued collaboration with the Clinical Center staff in various research projects and the development of new and improved techniques within the laboratory are an important part of the future program.

2. A continuation of the wound washing and blood study by quantitative techniques will be continued in collaboration with the Surgery Branch.

Part B included Yes (x) No ( )

Part B.

Publications other than abstracts from this project:

Del Vecchio, Pasco R., DeWitt, S. H., Borelli, J. E., Ward, J. B., Wood, T. A., Jr., and Malmgren, R. A. Application of Millipore Filtration Technique to Cytologic Material. J.N.C.I. 22: 427-431, 1959.

DeWitt, Sara H., Rabson, Alan S., Legallais, Frances V., Del Vecchio, Pasco R. and Malmgren, Richard A. Chromocenters Resembling Sex Chromatin in Human Tissue Culture Lines. J.N.C.I. 23: 1089-95, 1959.

Malmgren, Richard A. and Potter, John F., Cancer Cells in the Circulating Blood. Southern Medical Journal. 52: 1359-62, 1959.

Honor Awards and Memberships:

1. American Medical Association Award of Merit for Exhibit, "Tumor Cells in the Blood" by Drs. Potter, Kaiser, Malmgren, Hilberg and Pruitt.

2. Incentive award to Mr. Pasco R. Del Vecchio and Mr. Philip R. Joram of the Scientific Reports Branch, Division of Research Services for development of Millipore Filter Holder, October, 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NCI 853
1. Pathologic Anatomy Branch
2. Surgical Pathology and Postmortem Service
3. Bethesda 14, Maryland

Part A.

Project Title: The Department of Pathologic Anatomy (Pathologic Anatomy Branch, NCI) has no specific research projects. Its diagnostic services are designed to serve the investigative efforts of the N.I.H. staff responsible for patient care. In many instances, investigative study of pathological material is of great importance to clinical research.

Staff:

* Dr. Harold L. Stewart, Chief, Pathologic Anatomy Branch
* Dr. Louis B. Thomas, Head, Surgical Pathology and Postmortem Service
* Dr. Richard A. Malmgren, Head, Cytodiagnosis Service
* Dr. John H. Edgcomb, Pathologic Anatomy Branch
* Dr. Alan S. Rabson, Pathologic Anatomy Branch
* Dr. Edward Price, Laboratory of Pathology, NCI
* Dr. Elizabeth W. Chu, Pathologic Anatomy Branch
+ Dr. Thomas F. Dutcher, Resident, Pathologic Anatomy Branch
+ Dr. R. Gerald Suskind, Resident, Pathologic Anatomy Branch
+ Dr. Martin Hicklin, Resident, Pathologic Anatomy Branch
+ Dr. Jack T. Lasersohn, Resident, Pathologic Anatomy Branch
+ Dr. William C. Roberts, Resident, Pathologic Anatomy Branch
+ Dr. Seymour M. Sabesin, Resident, Pathologic Anatomy Branch
° Dr. Leon Sokoloff, Laboratory of Pathology and Histochemistry, NIAMD
° Dr. Harold R. Stanley, Oral Pathologist, Clinical Investigations, NIH
° Dr. Igor Klatzo, Head, Clinical Neuropathology Section, NINDB
° Dr. Herschel Sidransky, Laboratory of Pathology, NCI

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* - These Pathologists constitute the staff of the Department of Pathologic Anatomy, Clinical Center (Pathologic Anatomy Branch NCI) and spend a major portion of their time in the service and diagnostic work of the Department.

\+ - These physicians are full time Residents in the Department of Pathologic Anatomy.

° - These associate pathologists spend part time in the activities of the Department of Pathologic Anatomy. In addition, several pathologists on the staff of the Laboratory of Pathology, NCI; i.e. Drs. Swarm, Dawe, Banfield, O'Gara, Stanton and Love, are available for consultation with the staff of the Department of Pathologic Anatomy.

Project Description:

No specific research projects. The service functions of the Department during the twelve month period, November 1958 through October 1959 included:

a) 265 autopsy examinations. On page 4 there is a table which shows the number of autopsies performed for each Institute. The residents perform nearly all of the postmortem dissections under supervision of the staff. A gross review is held by the staff and residents each morning at 8:30. The residents review the microscopic slides of each autopsy with one of the staff before completing the autopsy protocol.

The staff assists the residents in preparing for the numerous clinical conferences in which the Department participates.

b) 2601 specimens were accessioned in the Surgical Pathology Section. A complete listing of these specimens, by Institute, is given in the accompanying table. These specimens are initially examined and described by a resident and their reports are checked by the staff. Associate pathologists from other laboratories or sections are frequently consulted about problems in diagnosis in surgical pathology and/or assume responsibility for handling certain tissues removed for specific research purposes.

c) Cytodiagnosis Service: The report for this service is submitted separately.

The objectives of the Department of Pathologic Anatomy in the Clinical Center are twofold: first, to furnish a diagnostic service and second, to aid from a morphological standpoint the various clinical research problems which are under study in the Clinical Center. In the first 2 or 3 years after the Department was established, most of the time of the staff was taken up by the diagnostic responsibilities. During the past year, especially, the staff has been able to spend more time in collaborative research on Clinical Center patients with various members of the Clinical staff.

The methods used include those standard methods used in describing, fixing and sectioning various human organs and tissues. A wide variety of special staining procedures are used.

Distribution of human organs and tissues:

At present there are approximately 30 scientists in the various laboratories at N.I.H. who have requested particular tissues from surgical and postmortem services. On many occasions it is possible to furnish these investigators with fresh human material. A list of these investigators is kept by the secretarial staff and telephone calls are made to the various investigators whenever surgical or postmortem tissues are available.

Conferences:

The staff and residents take an active part in numerous Clinical Center and Institute conferences. In addition, there are several conferences regularly held in the Department including a surgical pathology conference, brain cutting conference and joint pathologists conference.

Part B included Yes (x) No ( )

REPORT OF PATHOLOGIC ANATOMY DEPARTMENT

INSTITUTE AND TYPE OF PATIENT

	NCI		NHI		NIAMD		NIAID		NIMDB		NIMH		NIDR		REF		TOTAL	
	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU
Postmortem-Pathologic Anatomy Service																		
Autopsies performed	161		61		15		12		13						3		265	
Blocks of tissue	11128		3808		969		827		621						105		17458	
Slides	25214		9289		2773		1955		1506						230		40967	
Slides actually stained	13274		4546		1338		1096		801						142	26	21197	26
Surgical Pathology																		
Specimens	1590		305		175		251		96				138		46		2601	
H-E staining	6200		1092		883		572		368				194		130		9439	
Frozen Sections	159		90		22		5		9								285	
Special Procedures	1080		430		188		480		140				4		81		2403	

Part B.

List of Publications and Collaborative Investigative Projects:

A. Dr. Louis B. Thomas:

1. Boggs, David R., Frei, Emil III, and Thomas, Louis B. Clostridial Gas Gangrene and Septicemia in Four Patients with Leukemia. New England J. of Med. 259: 1255-1259, Dec. 25, 1958.

2. Fox, Frederick, Davidson, John and Thomas, Louis B. Maturation of Sympathicoblastoma into Ganglioneuroma: Report of Two Patients with 20 and 46 Year Survivals Respectively. Cancer 12: 108, Jan.-Feb. 1959.

3. Fritz, Richard D., Forkner, Claude E., Jr., Freireich, Emil J., Frei, Emil III, and Thomas, Louis B. The Association of Fatal Intracranial Hemorrhage and "Blastic Crisis" in Patients with Acute Leukemia. New England J. of Med. 261: 59, July 9, 1959.

4. Paton, David and Thomas, Louis B. Simultaneous Occurrence of Primary Malignant Melanomas of the Eye and the Skin. A.M.A. Archives of Ophthalmology. 62: 645-652, October 1959.

5. McFarland, James; Thomas, Louis B., Gilbert, Joseph and Morrow, Andrew. Myocardial Necrosis Following Elective Cardiac Arrest Induced with Potassium Citrate. To be published in the J. of Thoracic Surgery.

6. Freireich, Emil J., Thomas Louis B., Frei, Emil III, Prine, M.D., Forkner, C.E. A Distinctive Type of Intracerebral Hemorrhage Associated with "Blastic Crisis" in Patients with Leukemia. Cancer. In press. January 1960.

7. Moore, Edward W., Thomas, Louis B., Shaw, Richard K. and Freireich, Emil J. Central Nervous System in Acute Leukemia: A postmortem study of 117 consecutive cases with particular reference to hemorrhage, leukemic infiltration, and the syndrome of meningeal leukemia. A.M.A. Arch. of Int. Med. In press.

8. Clinical Center Staff Conference at National Institutes of Health Moderator: Von Sallmann, Ludwig. Discussants: Kaufman, Herbert E., Haase, Gunter; Bartter, Frederick C., and Thomas, Louis B. To be published in Annals of Internal Medicine.

9. Kaufman, Herbert E. and Thomas, Louis B. The Vitreous Opacities Diagnostic of Familial Primary Amyloidosis. To be published in Annals of New England J. of Med. Dec. 17, 1959.

List of Publications and Collaborative Investigative Projects (Continued):

10. Hilbish, Theodore F., Besse, Byron E., Jr., Lusted, Lee B., Daves, Marvin L., Thomas, Louis B., and Forkner, Claude E., Jr. Acute Leukemia: Skeletal Manifestations in Children and Adults. A.M.A. Arch. of Internal Medicine 104: 741, Nov. 1959.

11. Current Projects which will lead to Publications:

1. Cancer of the Uterus. Dunham, Lucia J., Casper, Julian, Dorn, Harold F., Thomas, Louis B., Edgcomb, John H., Symeonidis, Alexander and Stewart, Harold L.

2. Study of Clinical Center Patients with Carcinoma of Cervix Treated by Pelvic Exenteration with Dr. Robert Smith, Dr. John Dillon and Dr. Jack T. Lasersohn.

12. Lectures:

1. Aspiration Biopsy. Lecture - demonstration with Dr. Alan S. Rabson as part of short course in surgical pathology, International Academy of Pathology, Boston, Mass., April 20, 1959.

2. Moderator - Clinicopathologic Conference, Annual Scientific Assembly, Medical Society of the District of Columbia, November 25, 1959.

B. Dr. John H. Edgcomb:

See separately submitted Annual Report.

C. Dr. Richard A. Malmgren:

See separate report for Cytodiagnosis Service.

D. Dr. E.B. Price, Jr.:

1. Kinsey, D.L., Price, E.B., Jr., and Smith, R.R. Effectiveness of Chemotherapy in Relation to Extravascular Invasion by Emboli Tumor Cells. Accepted for publication in Cancer.

E. Dr. Thomas F. Dutcher:

1. Dutcher, T.F. and Fahey, J.L. The Histopathology of the Macroglobulinemia of Waldenström. J.N.C.I. 22: 887-917, 1959.

2. Zubrod, Charles G., Hilbish, T.F., Fahey, J.L., Dutcher, T.F., Fox, Frederick A., Osserman, Elliott F. Waldenström's Macroglobulinemia: CPC at the National Institutes of Health. Ann. Int. Med. 50: (4), 1010-1027, April 1959.

3. Current Projects which will lead to Publications:

1. Edgar, E. and Dutcher, T.F. The Histopathology of the Bing-Neel Syndrome.

2. The Syndrome of occult gastrointestinal loss of plasma proteins Waldmann, Thomas A., Steinfeld, Jesse L., Dutcher, Thomas F., Davidson, John D. and Gordon, Robert S., Jr., will be presented at the American Gastroenterological Association meetings in the Spring of 1960.

3. The Immunocytochemical Demonstrations of Intranuclear Localization of 18S Gamma Macroglobulin in the Macroglobulinemia of Waldenström.

F. Dr. Martin D. Hicklin:

1. Marsh, H.H. and Hicklin, Martin D. Survey of Staphylococcal Types in Hospitals in the District of Columbia, Maryland and Virginia. In press. Medical Annals of the District of Columbia.

2. Demonstration of Bacteriophage Typing of Staphylococci. Annual Meeting of Maryland Society of Pathologists, Baltimore, Md. October 24, 1959.

3. Projects:

1. Incidense of Multiple Cancers in NIH Autopsies, 1953-1957, with Mr. Don Loveland, Statistician, NCI.

2. The Ovarian Response to Human FSH with Dr. Louis S. Thomas and Dr. Mortimer Lipsett, NCI.

G. Dr. R. Gerald Suskind:

1. CPC on toxoplasmosis with Dr. Jacobs and Dr. Kayoe will be published in Annals of Int. Medicine.

H. Dr. Seymour M. Sabesin:

1. Current Projects which will lead to Publications:

1. Electron Microscopy -- Learning techniques of Electron microscopy in laboratory of Dr. William Banfield. Will [illegible] begin project under Dr. Banfield on Electron Microscopy of collagen in various diseases, i.e., rheumatoid arthritis.

2. Case Reports to be written:
   1. Congenital heart with multiple defects, situs inversus and agenesis of spleen.
   2. Disseminated Cryptococcosis in a patient with Hodgkin's Disease.

I. Dr. R. C. Boylan:

1. Report of vascular lesions in two patients with dermatomyositis in collaboration with Dr. Leon Sokoloff.

2. Assisting Dr. Louis B. Thomas in pathological study of hamsters infested with schistosomiasis.

J. Dr. Elizabeth Chu:

See separate report from Cytodiagnosis Service.

K. Dr. Alan S. Rabson:

1. Rabson, Alan S. and Legallais, F.Y. Cytopathogenic Effect Produced by Polyoma Virus in Cultures of Milk-Adapted Murine Lymphoma Cells (Strain P388D1). Proc. Soc. Exp. Biol. and Med. 100: 229-233, 1959.

2. R. Love and Rabson, A.S. Cytochemical Studies of Milk-Adapted Murine Lymphoma Cells (Strain P388D1) Infected with Polyoma Virus. J.N.C.I. In press.

3. Malmgren, R.A., Rabotti, G., and Rabson, A.S. Intracellular Localization of Polyoma Virus Antigen Demonstrated with Fluorescein Labeled Antisera.

4. Rabson, A.S. and Kirschstein, R.L. Intracranial Sarcomas Produced by Polyoma Virus in Syrian Hamsters. Archives of Pathology In press.

5. Rabson, A.S. and Legallais, F.Y. Growth of Human Epidermoid Carcinoma in Tissue Culture Using Nonfat Milk Medium. Archives of Pathology 68: 342, 1959.

6. DeWitt, Sara H., Rabson, Alan S., Legallais, Frances Y., Del Vecchio, Pasco R., and Malmgren, Richard A. Sex Chromatin in Human Tissue Culture Cell Lines. J.N.C.I. In press.

L. Current Projects which will lead to Publications:

1. Morphological studies of patients with cryoglobulinemia in collaboration with Dr. John Fahey and Dr. Paul Schwab.

2. Morphological studies of patients with hyperparathyroidism in collaboration with Dr. Fred Bartter and Dr. Robert Smith.

L. Dr. J.T. Lasersohn:

1. Projects:

1. The function of the eosinophil -- Experimental procedure: Eosinophil response in blood of guinea pigs is being compared during active and passive anaphylaxis. In collaboration with Dr. Richard Malmgren.

M. Dr. William C. Roberts:

1. Projects:

1. Report of case: Mumps myocarditis with Dr. Sam Fox.

2. Idiopathic medial cystic necrosis - report of case.

N. Dr. Katherine M. Herrold:

See separately submitted Annual Report.

O. Dr. Robert Peters:

Morrow, Andrew; Peters, Robert; Waldhausen, J.A., Blesdwell, R.D. and Braunwald, E. Supravalvular aortic stenosis: Clinical hemodynamic and pathologic observations. Circulation. In press.

Serial No. NCI 500
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Histogenesis and Pathology of Induced and Spontaneous Tumors of Laboratory Animals

Principal Investigator: Harold L. Stewart

Other Investigator: Katharine C. Snell

Cooperating Units: 1. Dr. David Johnson, NIAMD
2. Dr. John B. Calhoun, NIMH

Man Years (calendar year 1959):
Total: 6 1/3
Professional: 2
Other: 4 1/3

Project Description:

Objectives: To gain knowledge of the etiologic and pathologic factors in the production of neoplasms in experimental animals and to make this information available to other investigators.

Methods: 1. Adrenal cortical carcinoma 494, a transplantable, hormonally-active tumor that originated in the adrenal cortex of an Osborne-Mendel rat, has been studied during the course of 12 generations of transplantation in intact, mature hosts. Chemical extraction and paper chromatography studies of the tumor tissue are being made by Dr. David Johnson. A subline of this tumor, designated 494H, that developed when tumor 494 was grown for one generation in hypophysectomized rats and then returned to intact mature rats, has been studied for 6 generations. Transplantation of tumor 494 to hypophysectomized rats is being repeated to determine whether transformation to a tumor similar to 494H will occur again. In collaboration with Dr. R. C. MacCardle a paper dealing with the cytologic changes in the kidneys of rats bearing tumor 494 is being prepared.

2. A study of the spontaneous neoplasms occurring in the Mastomys, a South African rodent, is being continued. To date 119 of these rodents have been necropsied, and tissues from about half of them have been examined histologically. Several tumors have been transplanted to Mastomys pre-treated with various doses of irradiation, since transplantation of tumors to untreated Mastomys has been entirely unsuccessful.

3. A long-term study of the effect of age upon untreated rats of various ages is being continued. Spontaneous tumors occurring in these rats are being enumerated and classified.

Part A (continued)

4. Monkeys that received injections of carcinogenic substances into the wall of the glandular stomach approximately 10 years ago are being necropsied and studied to ascertain whether these substances may have produced gastric carcinoma.

5. Collaborative work with Dr. Harold P. Morris: Approximately 4000 histologic sections from rats fed either di-acetylaminofluorene or 2,7-fluorenylenebisacetamide have been examined. A manuscript is being prepared on the effects of these two compounds upon various organs of the treated rats, and separate manuscripts are being written on unusual lesions of the salivary glands, cranial nerves, and glandular stomach.

6. Collaborative work with Dr. John B. Calhoun: Experiments of Dr. Calhoun suggest that rats living under a certain amount of stress in a free-ranging community develop more tumors at an earlier age than do rats living under the usual laboratory conditions. We have necropsied a number of these rats and are studying the tissues histologically.

Major Findings: 1. During 12 generations of transplantation into intact, mature, male and female Osborne-Mendel rats Adrenal cortical carcinoma 494 has maintained a consistently similar histologic pattern of cords of uniform polygonal cells. All tumor-bearing rats have developed polyuria, polydipsia, and degenerative changes in the distal convoluted tubules of the kidney and have shown atrophy of the adrenal glands, sex organs, lymphatic tissue, and pituitary gland. Preliminary analyses of tumor tissue by Dr. Johnson indicate that this tumor secretes an excess of steroid hormones at least similar to those present in the normal adrenal cortex. A subline, tumor 494H, developed after transplantation of tumor 494 into hypophysectomized rats, differed in histologic pattern from tumor 494 in that it contained large, bizarre cells in addition to the uniform, polygonal cells of tumor 494. When tumor 494H was returned to intact, adult male and female rats, it retained its own distinctive histologic pattern, yet it also induced polyuria, polydipsia, and renal tubular degeneration. Rats of both sexes inoculated with tumor 494H developed hyperplastic mammary glands filled with milky secretion. In the females, the uterine horns were enlarged and the vaginal epithelium mucified; in the males, the testes and accessory sex organs were extremely atrophied. A few inoculated with tumor 494H have developed mammary carcinoma in addition to hyperplasia of the mammary tissue. This tumor induces development of the secondary sex organs in gonadectomized and in hypophysectomized rats but not of the testes or ovaries.

2. Tumors found in Mastomys have included a few carcinomas of the glandular stomach as well as papillomas of the forestomach, lung tumors, adenomas of the adrenal cortex, granulosa cell tumors and luteomas of the ovary, and lymphomas. The incidence of hepatomas has been unusually high.

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Individual Project Report
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Part A (continued)

3. No significant findings.

4. No significant findings.

5. Rats fed the carcinogen, 2-7 fluorenylenebisacetamide by Dr. H. P. Morris and examined histologically in this laboratory have shown an unusual number and variety of neoplasms. Among these lesions are: histologically malignant carcinoma of the glandular stomach, adenocarcinoma of the small intestine, severe atrophy and sarcoma of the salivary glands, neurogenic sarcoma originating in the region of the trigeminal nerves and pituitary gland, adenocarcinoma of the uterus. Carcinomas of the liver, ear, and mammary gland are also present in a high percentage of rats fed this compound.

6. Histologic study of rats from the experiment of Dr. Calhoun has not been completed. Preliminary data indicates that probably the tumor incidence in his stressed rats is little if at all higher than in rats living under normal laboratory conditions.

Significance: The knowledge gained from a study of neoplasms in laboratory animals should provide a basis for further work on the mechanism of carcinogenesis in human beings.

Proposed Course: Study of Adrenal cortical carcinoma 494 and of its subline, 494H will be continued, and it is expected that in collaboration with Dr. Johnson determinations of the hormones produced by these tumors will be completed. Collaborative work with Dr. MacCardle on the cytopathology of mitochondrial batonnets in the renal tubules of polyuric rats bearing adrenal cortical tumors will be continued, and a manuscript on the subject should be ready for publication during 1960. Collaborative work with Dr. Morris is expected to be ready for publication during 1960. The experimental procedures and examination of histologic material mentioned in other items enumerated above will be continued.

Part B included: Yes

Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Stewart, H. L., and Snell, K. C.
The histopathologic of experimental tumors of the liver of the rat. Chapter IV in The Physiopathology of Cancer (F. Homburger, ed.) Paul B. Hoeber, Inc., New York, 1959.

2. Stewart, H. L.
The Cancer Investigator.
Cancer Res. 19: 804-818, 1959

3. Snell, K. C., and Stewart, H. L.
Variations in Histologic Pattern and Functional Effects of a Transplantable Adrenal Cortical Carcinoma in Intact Hypophysectomized, and Newborn Rats.
J. Nat. Cancer Inst. 22: 1119-1155, 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NCI 502
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Md.

Part A.

Project Title: Histogenesis and histopathology of cutaneous neoplasms.

Principal Investigator: Dr. John H. Edgcomb

Other Investigators: None

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: -

Patient Days (calendar year 1959):

Project Description:

A. Study of diseases in man (collaborative research).

i. Mycosis fungoides

Ten patients have expired and have been autopsied. Two articles have finally appeared in print (see publications and enclosed reprints). A larger pathological article is contemplated to include the clinical and pathological observations made in the ten necropsied cases.

ii. Melanomas

The study of naevi and melanomas continues. We now have autopsied fifty patients with advanced melanoma. Dr. Rothberg and I have isolated a small quantity of black material from the urine of a melanuric patient. This material will be studied by physical and chemical methods.

One woman with a melanoma excreted large amounts of gonadotrophic hormone in her urine. The significance of this finding is being studied.

iii. Because of insufficient material the xeroderma pigmentosum study has been discontinued for the present.

B. Study of diseases in animals

i. Four melanomas have been produced in 51 guinea pigs by skin painting with dimethylbenzanthracene. This experiment has been completed.

ii. The hairs of chinchillas are being examined for the periodicity of melanin production in these animals. Chinchillas have an agouti coat, and the pattern of melanin production in hair appears to be very regular but very complex.

C. Miscellaneous collaborations and efforts

1. With Dr. R. Love, NCI.

I am reading slides on C3H mice injected at birth with the SE polyoma virus. The animals are over one year old, and this experiment continues as an effort to detect any late effects of the infection.

2. With Dr. R. Hertz, NCI.

Dr. Hertz and I have agreed to prepare a descriptive paper correlating the pathological and clinical observations on those patients with adrenal cortical carcinoma who have been autopsied.

3. I am studying necropsies of six adults and one child who died with cytomegalic inclusion disease.

4. I have helped Dr. L. Dunham in drafting small parts of her monograph on the cervical cancer study.

Part B included Yes (x) No ( )

Part I

Honors:

Lectures in pathology, Howard University

Appointment as member of a mission of government pathologists to Russia.

Publications:

Faulkner, C.E., Jr., Frei, Emil III, Edgcomb, John H., and Utz, John. Pseudomonas septicemia. Amer. J. Med. 25: 877-890, 1958.

Edgcomb, J.H., Van Scott, E.J., and Andrews, J.R. Histopathologic changes in the skin of patients with mycosis fungoides following therapy with high energy electrons. Acta dermat.-venereol., Proc. 11th Internat. Congr. Dermat. 1957, Vol. II, p. 457-462.

Van Scott, E.J., Andrews, J.R., and Edgcomb, J.H. Therapy of mycosis fungoides with high energy electrons. Acta dermat.-venereol. Proc. 11th Internat. Congr. Dermat. 1957, Vol. II, p. 451-456.

Cramer, L.M., Waite, J.H., Edgcomb, J.H., Powell, O.C., Tuohey, J.H., Van Scott, E.J. and Smith, R.R. Burn following accidental exposure to high energy radiation. Ann. Surg. 149: 286-293, 1959.

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NCI 525
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda 14, Maryland

Part A.

Project Title: Attempt to induct carcinoma of the bladder in hamsters by infection with Schistosoma haematobium.

Principal Investigator: Dr. Eloise Cram.

Other Investigators: Dr. Louis B. Thomas, Dr. Elmer Berry and Dr. Louis Olivier.

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 1
Professional: 1
Other: -

Project Description:

This research project is in collaboration with Doctors Eloise Cram, Elmer Berry and Louis Olivier, N.I.A.I.D. and consists of studying chronic infection with S. haematobium (Gold Coast strain and Egyptian strain) in hamsters; particularly with reference to the induction of bladder carcinoma.

a.) 217 hamsters were exposed to approximately 300 cercariae (Gold Coast strain S. haematobium) each early in 1955. Practically all of these hamsters have now died and have been autopsied. Histological sections have been prepared.

b.) Late in 1956 and during the first six months of 1957, S. haematobium from Egypt became available through the efforts of Drs. Berry and Olivier. Approximately 150 hamsters were exposed to cercariae and approximately 80 of these animals are still living.

Thus when this experiment is completed it will be possible to compare chronic S. haematobium infestation in the two groups of animals with Egyptian and Gold Coast strains of S. haematobium.

Significance to Cancer Research:

S. haematobium infection has been found associated with bladder carcinoma in several parts of the world. This study is designed to produce bladder cancer in the hamster by chronic infection with S. haematobium. The incidence of bladder cancer associated with Schistosomiasis is high in Egypt and relatively low in the Gold Coast. The study is also designed to determine whether there are pathological differences between infestation due to S. haematobium from these two parts of Africa.

Part A (continued)

Proposed Course of the Project:

All but approximately 30 of the animals have now expired after living from two to nearly three years following infestation with S. haematobium. Grossly, no bladder tumors have been observed. During the next year histologic sections of the autopsied animals will be performed so that this experiment can be completed.

Serial No. NCI 532
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cytochemistry of Ribonucleoproteins

Principal Investigator: Robert Love

Other Investigators: Dr. T. P. Bharadwaj (B,C,D,E,L)
Dr. R. G. Sushind (A) Principal Investigator (K)
Dr. N. P. Salzman (F) (NIAID)
Dr. A. J. Dalton (A)
Dr. M. O. Ellen (Wistar Institute) (K)
Dr. H. Kobayashi (E,I)
Dr. L. Kilham (J) (DBS)

Cooperating Units:

Man Years (calendar year 1959):
Total: 1 1/3
Professional: 2/3
Other: 2/3

Project Description:

Objectives: To study the ribonucleic acid metabolism of normal, neoplastic and virus-infected cells.

Methods: A. Further development of new staining procedures for ribonucleoproteins.

B. Study of the cytochemistry of ribonucleoproteins in rat liver cells during starvation and re-feeding.

C. Investigation of the structure and function of the nucleolus.

D. Development of a simple stain for mitochondria.

E. Study of the ribonucleoprotein metabolism of Ehrlich ascites cells accompanying the mitotic abnormalities produced by colchicine.

F. Cytochemical and biochemical stains of HeLa cells infected with a DNA virus (vaccinia).

G. Cytochemical studies of HeLa cells infected with a new (probably RNA) virus, HA-1.

Part A (continued)

H. Cytochemical studies of oncolysis produced by Newcastle Disease Virus in the Ehrlich ascites tumor.

I. Attempts to inhibit mitosis by treatment of Ehrlich ascites tumor cells or D98OD1 tissue culture cells with ribonuclease.

J. Study of the cytochemistry of squirrel and rabbit fibroma virus infection.

K. Investigation of the nature and properties of a ribonuclease contaminant of bovine testicular and streptococcal hyaluronidase.

L. Attempts to modify RNA metabolism by treatment of Ehrlich ascites cells with azaserine and 2-deoxyglucose.

Major Findings: A. 1. The chemical basis of the toluidine blue-molybdate (TBM) method of staining nucleoproteins has been determined. 2. In addition to the seven forms of RNA-protein originally delineated by the TBM method, two other forms of RNA-protein have been recognized. 3. It has been shown that electron-dense molybdate is deposited at sites of metachromatic staining by the TBM method.

B. The changes in the ribonucleoproteins of the rat liver have been studied by the TBM method. Some forms of RNP were unaffected by starvation; others disappeared and reappeared after re-feeding. The results suggested that some of the cytoplasmic RNP was formed in the nucleus.

C. The nucleolinus has been observed in a number of normal and neoplastic cells. The nucleolinus was usually large in neoplastic cells and was definitely increased in size in cells infected with Newcastle disease virus, in liver cells during re-feeding after starvation, in cells prevented from entering prophase by colchicine and in regenerating rat liver cells fed ethionine (by courtesy of Dr. H. Sidransky).

D. A simplified iron-hematoxylin stain suitable for use on blocks fixed in formol-sublimate has been developed.

E. Depending on the dose, colchicine produced either metaphase arrest or complete inhibition of mitosis of Ehrlich cells. In the former instance, nuclear parachromatin failed to diffuse into the spindle area at the end of prophase. In the latter case, where the onset of mitosis was inhibited, there was an increase in the size of the nucleolus and nucleolinus and an increase in the parachromatin of the interphase cells. The results suggest that the diffuse nuclear parachromatin may play a role in the genesis of the spindle fibers.

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Part A (continued)

F. The development of motile vaccinia virus particles has been observed under the phase microscope. Granular RNP, as well as DNA was associated with the formation of the cytoplasmic inclusions produced by vaccinia virus. The cytochemical studies have been correlated with virus titration and biochemical analyses of the nucleic acids of the cell.

G. Preliminary studies indicated that there are marked differences between the cytopathogenic effect of HA-1 virus on monkey kidney and on HeLa cells. A number of cytopathogenic effects that have not been described before have been observed. See. Dr. Sushkind's report.

H. A study of the cytopathology of Ehrlich ascites cells infected with Newcastle Disease Virus has been published.

I. Ribonuclease, even in doses of 40 mgm/ml., had no significant effect on the growth or RNP metabolism of any of the cells tested.

J. Material from squirrel and rabbit fibromas has been collected but has not yet been studied.

K. All preparations of hyaluronidase tested contained ribonuclease. The properties of the contaminant ribonuclease have been elucidated and compare with those of crystalline pancreatic ribonuclease.

L. Amsacrine and 2-deoxyglucose treatment produced no detectable effect on the growth or on the nucleic acids of Ehrlich ascites tumor cells.

Significance: The role of RNA metabolism of the cell is of fundamental importance in the understanding of genetics, protein synthesis, cell growth and differentiation, neoplasia and virus infection.

Proposed Course: 1) Continued investigation of the cytochemistry of RNP. Development of the TRK staining procedure for electron microscopy. 2) Further studies of the nucleolinus and of RNP metabolism in various systems: (a) cells infected with DNA and RNA viruses using newly-developed techniques and autoradiography. (b) Cells in which protein synthesis or cell division is inhibited by various procedures.

Part B included: Yes

PHS-1MH
Individual Project Report
Calendar Year 1959

Part 2. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Love, R., and Bharadwaj, T. P.
Two types of ribonucleoprotein in the nucleolus of mammalian cells.
Nature 183, 1453-1454, 1959.

2. Love, R., and Liles, R. H.
Differentiation of nucleoproteins by inactivation of protein-amino groups and staining with toluidine blue and ammonium polysulfide.
J. Histochem. and Cytochem. 7, 164-181, 1959.

3. Love, R.
The cytopathology of virus-infected tumor cells.
Ann. N. Y. Acad. Sci. 81, 101-117, 1959.

4. Bharadwaj, T. P., and Love, R.
Cytology of rat liver cells during starvation and refeeding.
J. Nat. Cancer Inst. 23, 695-715, 1959.

5. Bharadwaj, T. P., and Love, R.
Demonstration of mitochondria in tissues fixed for cytochemical and general staining procedures.
Stain Technol. In press.

Serial No. NCI 533
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Viral Therapy of Tumors

Principal Investigator: Robert Love

Other Investigators: Dr. R. G. Suckind (C)

Cooperating Units:

Man Years (calendar year 1959):
Total: 1 1/3
Professional: 2/3
Other: 2/3

Project Description:

Objectives: To adapt viruses to become oncolytic for tumors.

Methods: A. Passage of Newcastle Disease Virus (NDV) through various tumors in vivo.

B. Analysis of the oncolytic effect of the virus.

C. Comparison of the oncolytic effect of rat-HeLa adapted and unadapted Coxsackie virus on HeLa cells in rats and chick embryos.

Major Findings: 1) NDV was adapted to infect and to destroy the Ehrlich ascites tumor. 2) The Ehrlich adapted virus grew poorly in the Krebs 2 tumor and produced no oncolysis. After 18 passages in vivo through the Krebs 2 tumor the Ehrlich-adapted NDV became oncolytic. It has not been possible to adapt the original chick embryo NDV to the Krebs tumor by in vivo passage. 3) Chick embryo NDV produced some oncolysis of the P388 mouse ascites tumor while the Ehrlich-adapted virus had no effect. After a few passages in vivo through the P388 tumor the chick embryo NDV became more definitely oncolytic while the Ehrlich-adapted virus was still without effect on tumor growth. 4) HeLa cells could not be grown with sufficient regularity to permit a study of the effect of Coxsackie virus on HeLa cells.

Significance: The adaptation of a virus to a given tumor does not confer oncolytic properties for other tumors. In some instances a virus may be adapted to one tumor may be easier to adapt to another. On the other hand adaptation to one tumor may have the opposite effect and render the virus more difficult to adapt to another tumor.

Item 4 (continued)

Proposed Course: a) Completion of the project of EMC and Ehrlich, Krebs 2 and P388 cells; b) an attempt will be made to adapt ascitic embryo EMC to the Ehrlich tumor without the use of tissue culture; c) further studies of virus-induced oncolysis will be made with spontaneous or virus-induced tumors.

Part B included: Yes

Item 8. Honors, Awards, and Publications

Publications other than abstracts from this project.

1. Love, R.
Introduction to the Conference on "The Cytopathology of Virus Infection."
Ann. N. Y. Acad. Sci. 81: 4-5, 1959.

2. Love, R.
Editor of above.
Ann. N. Y. Acad. Sci. 81: 1-813, 1959.

3. Love, R.
The cytopathology of virus-infected tumor cells.
Ann. N. Y. Acad. Sci. 81: 101-117, 1959.

Serial No. [illegible]
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Filtrable Agents from Mouse Tumors.

Principal Investigator: Robert Love

Other Investigators: John H. Edgcomb (A only)
Alan S. Rabson (B only)

Cooperating Units:

Man Years (calendar year 1959)
Total: 1 2/3
Professional: 2/3
Other: 1

Project Description:

Objectives: A. To propagate a filtrable agent from mouse leukemias in tissue culture.

B. To study the cytopathology of polyoma virus infection in tissue culture.

Methods: A. Extracts from a lymphocytic neoplasm were passed serially in monkey kidney tissue cultures and inoculated into C3H/HeJ x 129 and C3HF/ HeN x AKR newborn mice. Control mice were inoculated with extracts of normal tissues treated in the same manner.

B. P388D1 murine lymphosa cells grown in a milk medium were infected with polyoma virus and studied by various cytochemical techniques.

Major Findings: A. A variety of tumors were produced in mice by inoculation of the first passage monkey kidney material containing the leukemia extract.

B. In P388D1 cells infected with polyoma virus, the ribonucleoprotein of the nuclear parachromatin and the nucleolinus could not be demonstrated. The nucleolus was enlarged and vacuoles containing a filtrate and a condensed form of abnormal ribonucleoprotein appeared in the nucleus. The nuclei were at first enlarged but later shrank, became pyknotic and disintegrated. Nuclear fragments frequently underwent phagocytosis by other less damaged tissue culture cells. Deoxyribonucleic acid could not be demonstrated with certainty in association with the newly-formed abnormal ribonucleoprotein in the nucleus.

Part B (Continued)

Significance: The nature of the interaction between virus and host cell and the factors determining whether the outcome will be neoplastic or proliferation is of fundamental importance in the study of virus-induced neoplasia.

Proposed Course: A. All the surviving mice will be held on test for 18 months at the end of which time the results will be analyzed.

B. In view of reports that the infectious nucleic acid of polyoma virus is deoxyribonucleic acid new techniques will be applied to detect any newly formed DNA in the nucleus.

Part B included: Yes

Part X. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Love, R., and Mabson, A. S.
Cytochemical studies of milk-adapted murine lymphoma cells (strain P388D1) infected with polyoma virus.
J. Nat. Cancer Inst. November 1959.

Cal No. [illegible]
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A review of the surgical and autopsy sections from Veterans of World War I whose cause of death has been listed as lung cancer.

Principal Investigator: Katherine McD. Herrold

Other Investigators: Harold L. Stewart, Laboratory of Pathology, [illegible]
Harold F. Dorn, Biometry Branch, DRS, NIH

Cooperating Units:

Man Years: (calendar year 1959)
Total:
Professional:
Other:

Project Description:

Objectives: The objective of the study is to review all the cases from veterans of World War I which have been diagnosed as lung cancer and to determine whether the findings meet the criteria for primary lung cancer. The second objective is to classify the tumor according to the histological type.

Methods: This review is being done in conjunction with the epidemiological study of the National Cancer Institute on the relationship between smoking and death from carcinoma of the lung.

Pilot studies using Syrian hamsters are in progress to determine an experimental approach for studying the pathogenesis of lung cancer. Intratracheal instillation and transplantation of newborn tissue, including bronchi and lung tissue into weanling hamsters have been tried. Attempts to produce tracheal fistulae in hamsters so far have been unsuccessful.

Major Findings: This study has not been in progress a sufficient length of time for evaluation of the significant findings.

Significance: The knowledge gained from this review of lung cancer cases may elucidate the importance of proposed etiological factors.

Proposed Course: To continue reviewing the surgical and autopsy material sections from the cases of veterans of World War I and to perform experimental research relating to lung cancer.

Part B included: No

Serial No. NCI 536-a
1. Laboratory of Pathology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental studies relating to the minimal concentration of hydrocarbons which will produce cancer in hamster cheek pouches.

Principal Investigator: Dr. Katherine McD. Herrold

Other Investigators: None

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: -

Patient Days (calendar year 1959):

Project Description:

Objective:

The objective of the study is to determine the minimal concentration of known carcinogens which will produce cancer in hamster cheek pouches and to study the histolytic changes during the early initiations and promotion stages.

Methods:

Test pellets using C.P. beeswax as a vehicle and containing various concentrations were prepared. The hydrocarbons which are being used in the experiment are 9, 10, dimethyl - 1, 2 - benzanthracene and 10-methylcholanthrene. The pellets have been inserted in the right cheek pouch of 63 hamsters and will be left in situ for indefinite periods. Pellets consisting only of beeswax have been inserted into the cheek pouch of male and female hamsters and will serve as controls. The technique being used is the one developed by Dr. Lucia Dunham (see Serial No. NCI 501b).

Major Findings:

A marked difference has been observed between the action of 10 methylcholanthrene and 9, 10 dimethyl 1, 2 benzanthracene on the buccal membranes of the hamster cheek pouches. Concentrations of 2-3 mgs. and above of 9, 10 dimethyl 1, 2 benzanthracene produce acute inflammation, ulceration and necrosis of the hamster cheek pouch and within a period of 10 days to two weeks the pellet sloughs out.

Part A (Continued):

The end stage in many of these pouches is atrophy and in a few of the cases neoplastic changes have occurred. With lower concentrations of the carcinogens, epidermoid carcinoma has developed in many of the hamster cheek pouches so far examined. The livers of the tumor bearing animals reveal prominent hematopoiesis.

High concentrations of 20 methylcholanthrene have produced only negligible changes in the Hamster cheek pouches and no tumors to date have developed. The control animals which have been examined reveal no significant pathological changes in the pouches.

Proposed Course of Project:

Complete necropsy examinations are being performed on all animals and the pathological changes will be correlated and evaluated. The findings will dictate the future course of the experimental study.

Part B Included Yes (x) No ( )

Item B. Publications:

A case report has been prepared and the paper is ready to be submitted for publication.
TITLE: Aberrant Parathyroid Gland in Pharyngeal Submucosa.

Serial No. NCI 903
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Collaborative Research Requiring Pathologic Assistance

Principal Investigator: Thelma B. Dunn

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To collaborate with and advise other investigators at the National Cancer Institute in phases of research problems requiring experience in animal pathology.

Methods: This may consist of (1) Full responsibility and separate publication as senior author of material reviewed in connection with work of another investigator, (2) Review and description of animals coming to autopsy, and publication of the findings as a separate section of a paper describing the entire experiment, (3) Review and diagnosis of tumors found by investigator in course of experiments, the information to be used in a tabulation of results, (4) Occasional consultation and advice on pathologic aspects or material in an experiment.

Major Findings: 1. With Dr. Ruth Kirvin. Mice which had been implanted intraperitoneally with 'sandwiched' containing a variety of different tissues have been autopsied. The results have not been tabulated but a significant number of plasma cell neoplasms, diffusely seeded on peritoneal surfaces, have been found. Spontaneous tumors of this type have never been found. Plasma cell neoplasms of this type have only been found in a BALB/c male mouse which received no estrogen, in a C3H mouse, and in a mouse with a blank "sandwich." This eliminates several factors--sex, estrogen stimulation, BALB/c genetic constitution, and "foreign" tissue in sandwich--as necessary factors for the induction of the plasma cell neoplasms. Other neoplasms, particularly fibrosarcomas, have also been found but these usually appear in close association with the sandwich. Dr. Michael Potter and Dr. John Fahey are studying the abnormal proteins of these tumors, Dr. Jack Dalton possible virus particles, and Dr. Rose MacCardle the Golgi apparatus.

Individual Project Report
Calendar Year 1958

Part A (continued)

2. With Dr. Katherine Stanford. The morphology of tumors grown in the mouse from tissue culture cells.

3. Continued work with Dr. Andervont on mammary tumors.

4. With Dr. John Maloney on leukemia produced by a cell-free filtrate. DBA/2 mice were injected with spleen extracts from normal mice, and from "virus leukemia" mice. Autopsies have been performed at frequent intervals on mice from 2 groups. At present time (12 weeks after injection) no difference has been detected.

Significance: Pathologic review is indispensable to many types of cancer research. This is best carried out by a specialist in pathologic anatomy, since with the present complexities of research a single investigator cannot know this field and his own specialty.

Proposed Course: This work will continue as in the past. Then, as a result of many observations, enough information is accumulated regarding any subject, publications will be made.

Part B included: No

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A(1)

Project Title: An Electron Microscopic Investigation of Normal and Neoplastic Tissues.

Principal Investigator: William G. Banfield

Other Investigators: Dr. Dawe, Dr. Rabson, Dr. Ramirez, Dr. Longley, Darlene C. Brindley

Cooperating Units:

Man Years (calendar year 1959):
Total: 1 1/2
Professional: 1
Other: 1/2

Patient Days (calendar year 1959)

Project Description:

Objectives: 1. To describe and interpret the ultra structure of [illegible] neoplasms and to compare results with suitable control tissues.

2. To look for and study the inciting agent or accompanying virus in neoplastic lesions and compare with similar agents or viruses if known.

Major Findings: 1. The polyoma virus was described for the first time [illegible] section obtained from cultured cells supplied by Dr. Dawe [illegible] publication). A larger viral form probably different from the polyoma also present in this material. The same cells grown by Dr. Rabson [illegible] and infected with polyoma contained only the 300 A° polyoma particle and the larger particles. The 300 A° polyoma particles grow in cultures after the inoculum to 60° for 30 minutes.

2. Skin lesions were examined from malignant and [illegible] acanthosis nigricans and tumor from the malignant lesion. Control sections normal and jaundiced skin were also examined. Small particles 50-100A diameter were found in great abundance in the basal cells of the malig[illegible] acanthosis nigricans, less frequently in the benign and least in the control. Their exact identity has not been established but they are probably [illegible] as yet has not been described in skin. Dr. Melon Cork and her husband and Dr. Cronin made it possible for us to obtain these lesions. Dr. Dawe could grow nothing from these lesions in tissue culture.

3. Two lesions of molluscum contagiosum were obtained [illegible] Dr. Marc Wainer. The virus on gross visual examination has not grown [illegible] culture of keratinizing epidermal cells (Dr. Rabson) or on rabbit skin [illegible] rabbit testis.

4. In collaboration with Dr. Longley we have submitted a paper on the structure of the rete mirable in the kidney of the rat.

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Individual Project Report
Calendar Year 1959

Part A (1) (continued)

Significance: The viral nature of the polyoma agent was established beyond any reasonable doubt. The ultra structure of a new virus in the mammalian kidney, similar to that in the swim bladder of the fish has been described for the first time. A previously undescribed 50-100 A° dense granule in the basal layers of the skin has been found.

Planned Course: 1. With Dr. Kabasa and Dr. Dawe work out a life cycle of polyoma and attempt to find the significance of the "virus" particles in the serum cultures and their absence in the milk cultures.
2. Attempt to identify the 50-100 A° particles in the skin and the small dense granule in the molluscum elementary body.
3. Continue in the direction noted under project description.
4. In collaboration with Dr. Ramirez investigate the ultra structure of a lymphoma in the hamster and mycosis fungoides.

Project No. NCI 501a

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A(1)

Project Title: An Investigation of the Collagenous Connective Tissues and Elastin

Principal Investigator: William G. Banfield

Other Investigators: Darlene C. Brindley

Cooperating Units:

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 1 3/4
Professional: 1
Other: 3/4

Project Description:

Objectives: 1.To elucidate age changes and pathologic alterations in collagen and elastin.
2.To uncover mechanisms affecting the state of collagen in the body.

Major Findings: 1. In hamsters there is an increase in the abdominal skin collagen soluble in 1:3000 acetic acid between 18 and 21 days during the time of development of spermatogenesis and the appearance of many clear cells in the pituitary. Additional age changes have been noted but no definite correlations have yet been made.

2. As an outgrowth of the studies on ageing hamsters we have 9 transplantable tumors among which are two melanomas, a reticulum cell sarcoma, a lymphoma, 2 possible adrenal cortical tumors and an epidermoid carcinoma.

Significance: The data being collected on the hamster is yet to be completed. A fairly good base line for the normal development throughout the life span of the male hamster should be established.

Proposed Course: 1. Complete the histological portion of the ageing hamster program and analyze the data. Fill in the data with more old hamsters where needed.

2. In collaboration with Dr. Sabesin study the collagen from the foot of a patient with rheumatoid arthritis. This collagen shows many pathologic changes.

3. With the electron microscope and in collaboration with Drs. Sokoloff and Obrien, study the collagen in ochronosis. In addition collagen fibril changes in maturing scars, examine the collagen of cortisone treated hamsters and study elastic tissue in thin sections if a suitable method can be developed.

4. In collaboration with Drs. White, Reid and Jackson study the collagen at various sites in the rat for rate of decay of C^{14} labelled glycine from it is stopped after feeding from conception to about 4 months after birth.

Part B:

Honors, Awards, and Publications:

Publications other than abstracts from this project:

1. Banfield, W. G., and D. Brindley
An electron microscopic study of the epidermal lesion of molluscum contagiosum.
Ann. N. Y. Acad. Sci., 81: 103-163, 1959

2. Banfield, W. G.
Dense granule in the elementary body of molluscum contagiosum.
J. Biophysic. and Biochem. Cytol.,5: 513-514, 1959

3. Banfield, W. G., and D. Brindley.
Acetic acid-soluble collagen in human scars.
Surg. Gynec. and Obst. 109: 367-369, 1959

4. Banfield, W. G.
Age changes in the acetic acid-soluble collagen in human skin.
Arch. of Path. In press.

5. Banfield, W. G., Dawe, C. J., and Brindley, D. C.
Intracellular and extracellular particles in tissue cultures inoculated with extracts of human warts (Molluscum virus).
J. Nat. Cancer Inst. In press.

Journal No. NCI 505
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Induction of Squamous Carcinoma of the Lungs in Laboratory Animals

Principal Investigator: Mearl F. Stanton

Other Investigators: Robert Blackwell, Joseph Albrecht and staff

Cooperating Units:

Man Years (calendar year 1959):
Total: 5/6
Professional: 1/4
Other: 7/12

Project Description:

Objectives: To induce lung cancer in laboratory animals through deposition of known and suspected carcinogens in areas of regenerating lung tissue.

Methods: Certain aliphatic halocarbons are capable of causing ischemic necrosis when trapped in capillary vessels. By incorporating carcinogens such as methylcholanthrene into the inoculum it is possible to deposit the carcinogen in particular organs of interest to the oncologist. The detection of methylcholanthrene deposition by U.V. microscopy is being accomplished.

Major Findings: Intravenous administration of the halocarbon alone has not induced tumors in rabbits, rats, or mice, many under observation for one to two years. In mice and rabbits neither the halocarbon nor halocarbon + methylcholanthrene cause an increase in spontaneous alveologenic tumors nor do they induce carcinomas in the areas of infarction. In rats a suspension of methylcholanthrene in tricapoylin carried to the lungs by the halocarbon consistently induced metastesizing squamous carcinomas of the lung which kill the animals in six to seven months. The histogenesis of the tumors now under study indicates they arose in areas of bronchial metaplasia which result from halocarbon produced infarction. We have traced the deposition of methylcholanthrene with ultraviolet light microscopy.

Significance: Our studies may contribute to several major problems in the genesis of lung cancer in man such as the role of pulmonary scarring and its relationship to exogenous inhaled carcinogens which in themselves may act by injuring bronchial cells and subsequently altering their neoplastic potential. Such studies may also offer a new method for studying the mechanism of methylcholanthrene carcinogenesis.

Part A (continued)

Proposed Course: Study of the histogenesis of the tumors. Quantitative measurements of dose to response. Further testing as a result of scoring alone to induce carcinoma. Tests currently under study include the intravenous administration of infarct inducing halocarbons several days prior to and several days after intravenous methylcholanthrene in an effort to demonstrate the initiating and/or promoting effects of these substances. Further studies as outlined in Individual Project Report NCI 503, 1957, are also continuing.

Part B included: No

Serial No. TSI 506
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Transplanted Tumors in Mice

Principal Investigator: Thelma B. Dunn

Other Investigator:

Cooperating Units:

Man Years (calendar year 1959):
Total: 5/6
Professional: 1/3
Other: 1/2

Project Description:

Objectives: To study various unusual tumors which may throw light on the normal cell counterpart or cell function, and assist in understanding of neoplasia.

Methods: A number of tumor lines of unusual interest are now being carried. Among them are (1) Mast cell neoplasm, (2) Plasma cell neoplasms, (3) Functional adrenal cortical neoplasm, (4) Organoid tumor of uterus in BALB/c mice, (5) Osteogenic sarcomas.

Major Findings: (1) Mice bearing the mast cell tumor have been sent to a number of laboratories in the United States and also to Sweden. Tumor tissue has also been supplied to a number of investigators at the National Institutes of Health. The tumor in the ascites form is carried by Dr. Michael Potter, and in the solid form in this laboratory. Metachromatic granules are still abundant in the malignant cells after 5 years in transplant. Tumor tissue supplied from this laboratory has recently been used by Dr. Edward Korn of the National Heart Institute for studies of heparin.

(2) Several plasma cell lines have produced alterations in the kidney of tumor-bearing mice. These tumor lines have been supplied to Mr. Ralph Coleman, a medical student working under Dr. J. F. A. McManus at the University of Alabama Medical School.

(3) The adrenal cortical neoplasm incessantly produces virilizing changes in female mice, and other morphologic alterations

Part A (continued)

(4) Several tumors of the uterus are now carried in BALB/c mice. These are remarkably organoid, and one tumor, after seven months growth, produces a cystic cavity, lined by endometrial mucosa and stroma, and what appears to be a smooth muscle outer layer.

(5) Osteogenic sarcomas. These tumors often metastasize to the lungs and are being used in other laboratories for studies on tumor spread. A large multinucleated "osteoclastic" cell is regularly found in one of the tumors. In tissue culture (by Dr. Clyde Dawe) the multinucleated cell disappears but it reappears when transplanted back to mice.

Significance: Neoplastic alterations in cells which have easily recognized morphologic or metabolic characters may lead to a clearer understanding of neoplasia.

Proposed Course: The transplant behavior of these unusual tumors is being observed. Studies are planned to determine whether the uterine tumors respond to endocrine alterations.

Part B included: No

Serial No. NBI 507
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
Calendar Year 1959

Part A.

Project Title: Factors Involved in Abortive Mitosis in Human Hair Follicles in the Catagen (resting) Phase of the Growth Cycle

Principal Investigator: Rose C. MacCardle

Other Investigators: E. J. Van Scott

Cooperating Units:

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: We discovered that the epithelial cells of human hair follicles in the resting (catagen) phase of the hair growth cycle reveal evidence of arrested mitotic figures, whereas in the actively growing phase few if any such abortive mitotic figures could be found.

Methods: A microscopic study of biopsy specimens.

Significance: We are interested in determining whether basal and squamous cell tumors possess such figures. We are also interested in trying to determine whether some naturally occurring mitotic inhibitor may be present during the catagen phase and neutralized in the anagen phase,--a situation similar to the production of acetylcholine and its breakdown by cholinesterase.

Proposed Course: Finishing it for final proof, and we plan to attempt to extract a chemical agent from the catagen stage that would throw anagen into catagen.

Part B included: No.

Serial No. NCI 908
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cytology of Antibody-producing Plasma-cell Tumors of Mice and Human Multiple Myeloma

Principal Investigator: Ross C. MacCardle

Other Investigators: Michael Potter and John L. Fahey

Cooperating Units:

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: To determine the intracellular site of production of antibodies and to ascertain the cellular origin and fate of plasma cells.

Methods: By cytological techniques for Golgi apparatus mitochondria and RNA, we study the tumor tissue and also plasma cells induced to form in mice injected with rabbit serum or Freund's adjuvant.

Major Findings: The juxtanuclear clear cytoplasmic 'hof' by which the plasma cell is characterized consists of the Golgi apparatus and the centrosome. The plasma-cell tumors of mice possess three different types of Golgi apparatus consisting of: I. a few small strands; II. a diffuse network; and III. a large thick hypertrophied ball network. Two of the tumors producing beta-globulin can be distinguished from the others by their characteristic hypertrophied Golgi apparatus (Type III). However, the type of Golgi apparatus does not appear to be related to the electrophoretic mobility of the globulin, but rather to the speed of production of the protein. The cytoplasm of plasma cells is rich in RNA, but the Golgi area is free of it.

Plasma cells of plasma-cell tumors appear to derive from lymphocyte-like cells which in turn probably derive from undifferentiated lymphocytogenic cells of the germinal centers of lymph nodes.

Part A (continued)

Significance: Such information may lead to better understanding of the mechanism of production of antibodies and to a method of controlling the differentiation of plasma cells in plasma-cell tumors and multiple myeloma.

Proposed Course: We intend to pursue this problem in an attempt to prove the cellular origin of plasma cells.

Part B included: No

1
2. Cancer Induc. & Pat[illegible]
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Protoplasm of Tumor Cells: Characteristics of malignant neoplastic protoplasm

Principal Investigator: Ross C. MacCardle

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 5/6
Professional: 1/4
Other: 7/12

Project Description:

Objectives: This manuscript consists of about 300 typewritten pages being written in monograph form as part of an International Handbuch der Protoplasmaforschung edited by Heilbrunn and Weber. It describes the cytological and some physiological characteristics of tumor cells. It contains some new material such as plasma-cell tumors and pituitary tumors that has not been published elsewhere.

Proposed Course: To be sent to the printers on January 10. This will finish the work.

Part B included: No.

Serial No. NCI 510
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Endocrine and Reproductive Organs in the Mouse

Principal Investigator: Thelma B. Dunn

Other Investigators: Rose C. MacCardle is doing some phases of this study. Mr. Joseph Albrecht and Mr. Hans Bergman are making special preparations of the pituitary gland.

Cooperating Units:

Man Years (calendar year 1959):
Total: 5/6
Professional: 1/3
Other: 1/2

Project Description:

Objectives: This is a continuation of work begun several years ago. It is expected that it will continue for a considerable period of time since time is required for accumulation of animals. To accumulate reliable data by observations on untreated mice, planned procedures, and reviews of the literature regarding the reproductive and endocrine organs of inbred mice.

Methods: Personal observations on material submitted from different sources, planned experimental procedures in inbred mice, histologic study of untreated mice at various ages, systematic review of the literature.

Major Findings: This study is proceeding slowly because of pressure of other work. Considerable morphologic difference is found in the adrenal glands of inbred strains of mice. This material is already proving useful to other members of the staff since it represents a normal standard of some endocrine organs in the mouse. A beginning has been made on writing up results on the adrenal gland. The literature, which proved unexpectedly voluminous, has been reviewed. Amorphous particles seen in the medulla in Zenker-formol fixed adrenals are being studied by Dr. Sam Spicer. We has found similar particles in the adrenal medulla of other species. Mercuric chloride is required to precipitate them and no previous description has been found of these bodies. Location of the pineal gland of the mouse has also been determined, and some observations are being made.

Part A (continued)

Significance: Since hormonal studies are an important part of cancer research, it is desirable that reliable information be available regarding the endocrine and reproductive organs of inbred strains of mice.

Proposed Course: It is expected that this study will proceed slowly, and require several years for completion.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Dunn, Thelma B.
Cancer Research in the Soviet Union
J. Am. Med. Women's Assoc. 14: 314-316, 1959.

Dawe, C. J., Law, L. W., and Dunn, T. B.
Studies of Parotid Tumor Agent in Cultures of Leukemic Tissues of Man
J. Nat. Cancer Inst. 23: 717-797, 1959.

Honors and Awards relating to this project:

1. President, Washington Society of Pathologists
2. Member, Board of Directors, American Association for Cancer Research.

Serial No. PCI 511
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Collaborative Research

Principal Investigator: Richard L. Swarm

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To collaborate with and advise other investigators particularly in phases of research requiring experience in pathologic anatomy or the use of radioactive isotopes.

Methods: Methods vary widely and are dependent upon the problem and the extent of the collaboration.

Major Findings: Collaborations which have developed include the following: a) Collaboration with Dr. H. Morris on the effect of endocrine glands on the occurrence of hepatic neoplasms induced by 2-diacetylaminofluorine. Some progress has been made in the continuation of the histological study of hepatic tumors which were found to have developed more slowly in hypothyroid animals.

b) Collaboration with Dr. S. S. Spicer of the Laboratory of Pathology and Histochemistry (NIAMD) on a study of mucopolysaccharides. Through the study of tissues labeled with S^{35} it has been possible to confirm the findings of others that much of the acid mucopolysaccharides particularly of mucins are sulfated. In addition, however, some acid mucopolysaccharides were found to contain no S^{35}. Dr. Spicer has gone on to identify these non-sulfated acid mucins as those which have a high content of sialic acid. Presentation of this work was made at the Histochemical Society meeting, April 1959.

In addition as a result of past collaborations interest continues in: a) the distribution of tetracyclines and their fluorescent pattern in necrotic tissue and certain neoplasms; b) the metabolism of bilirubin and study of tissues from animals with a congenital enzymatic defect in the metabolism of bilirubin. This congenital defect is an inability to conjugate bilirubin and certain other chemicals and hormones with glucuronic acid.

Part A (continued)

During the past year the principal investigator has consulted with a number of scientists about their research work and has lectured in the three N.I.H. Isotope Training Courses given during the past year.

Significance: Collaboration is indispensable to many types of cancer research.

Proposed Course: Collaborations listed will be continued as desired.

Part B Included: No

Serial No. MET
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Transplantation Studies with Particular Emphasis on the Morphological Alterations of Cartilage Tissue and Endochondral Bone Formation

Principal Investigator: Richard L. Swarm

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To accumulate information about transplanted tissues with an emphasis on morphologic alterations of normal proliferating and senile cartilage tissue and neoplasms flowing transplantation.

Methods: Changes in the environment of cartilage tissue are effected by 1) auto, iso, homo or heterotransplantation; 2) alteration of the endocrine status of the host animal; 3) administration of material selectively retained in cartilage (S^{35}); and 4) by alteration of the diet (lathyrogenic compounds). This study is limited to experimental animals.

Interest in transplantation experiments in general exists and in order to better understand the transplantation of cartilage, study and collaboration in the transplantation of other tissues is made as opportunity develops.

Major Findings: Studies previously reported of iso- and homo-transplantation of a mixed tumor containing some cartilage tissue have been continued. Adaptation of this tumor to certain hybrids and homografts has been accomplished. Attempts to modify the amount of cartilage present in the tumor have not been very successful.

Selective proliferation or matrix formation of the cartilage in the homografted costochondral junction has been further studied using fetal and newborn cartilage as well as adult cartilage. These experiments were set up as chronic experiments and the only preliminary information available is that these grafts behave in a fashion similar to those previously studied.

Individual Project Report
Calender Year 1959

Part A (continued)

A pilot experiment with only very few animals has been started in which cartilage from a newborn animal was placed in tissue culture fluid containing polyoma virus and then homografted to adult animals. These animals are being observed for the development of neoplasms.

Dogs autografted intravenously with thyroid gland tissue by Dr. Richard J. Sanders were studied with Dr. John Edgcomb. Survival of the grafted thyroid tissue in and adjacent to pulmonary blood vessels has been demonstrated. Observation of surviving animals for chronic changes at the sites of the grafted thyroid tissue is being made.

During the past year studies were performed with a transplantable cartilagenous neoplasm in rabbits obtained from Dr. B. Levy of Houston, Texas. This tumor was transplantable by intraocular transplantation in Dr. Levy's rabbits. In our hands, newborn animals were transplanted with the tumor in the hopes that in the newborn decreased immunologic sensitivity and increased amounts of growth hormone might make possible greater growth. These subcutaneous transplants did increase in size initially but regressed, presumably due to a homograft reaction. Interestingly enough, all of the intraocular transplants we have tried have also been unsuccessful. One possible explanation of these results might be that the animals with which Dr. Levy has had success transplanting the tumor have all been closely related to each other. We are not anxious to have a tumor whose growth can be maintained only in the intraocular site. In an effort to effect subcutaneous or intramuscular transplantation of this tumor we have successfully transplanted tissue to fetal rabbits in utero. Further attempts at in utero transplantation are planned for this tumor.

Significance: This project developed as a result of collaboration with other investigators in the National Cancer Institute. It is basic in nature and of interest in understanding host-tissue responses.

Proposed Course: This project is to be continued.

Part B included: Yes

Part D. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Swann, R. L., Schatten, W. E., Sanders, R. J., and Bergenstal, D. M. The Autoradiographic Study of Transplanted Tissue. Laboratory Investigation 8: 149-161 (Jan-Feb. 1959)

Serial No. [illegible]
1. Laboratory of Pathology
2. National Insts. of Health
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Ontogenetic Development of Plasma Cells in Mice

Principal Investigator: Ross C. MacCardle

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959)
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: The lymph nodes, spleen, and ileocecal region of mice from fetus to old age are being examined to determine the age at which plasma cells first appear and to determine the rate of production at later stages. The cytological characteristics of plasma cells are being studied to ascertain the state of the Golgi apparatus, mitochondria and cytoplasmic RNA at different stages of maturation of the plasma cell. An attempt is being made to determine the cellular origin of plasma cells and to determine whether plasma cells can be formed under artificial stimulation prior to their normal date of appearance. It is not yet known whether each type of protein antibody is produced by a single [illegible] plasma cell, or whether all are produced in the same cell at different stages of maturation which may or may not be reversible.

Methods: Freund's adjuvent will be utilized as a stimulant to formation of plasma cells. This can be injected into the fetus as well as in the newborn. Tissues will be taken at various ages and fixed and stained cytologically.

Major Findings: In the mouse, plasma cells first appear in the lymphatic tissue of the intestinal mesentery at about ten days after birth. None were found before birth and none immediately after birth. Plasma cells at ten days of age (called tentatively puberty plasma cells) can be distinguished from those of the adult mouse by the size of their Golgi apparatus which is distinctly smaller than that of the adult plasma cells. It is not known whether such agents as Freund's adjuvant could induce the ten-day old mouse to form adult plasma cells, or whether a puberty plasma cell merely differentiates into an adult form.

Part A (continued)

Significance: These findings and the studies planned are believed to be of fundamental importance to an understanding of the genesis of a plasma-cell tumor in the mouse, and possibly also of multiple myeloma in man.

Proposed Course: It is intended to pursue this study, particularly with a view to determining the relative susceptibility of newborn and adult mice to the formation of plasma cell tumors.

Part B included: No

1. Laboratory of [illegible]
2. Cancer Endoc. & [illegible]
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Induced and Spontaneous Neoplasms in Laboratory Animals. Factors influencing their incidence.

Principal Investigator: Ambadas S. Mulay

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: A. Study the role of sex hormones on the incidence of hepatoma induced by azo dyes in male and female rats. B. Influence of primary and transplanted tumors on tissue and serum chemistry of rats.

Methods: A. Male and female Osborne-Mendel rats were fed on semisynthetic diet containing p-dimethylaminoazobenzene, p-dimethylaminoazobenzene-1-azo-1-naphthalene, p-dimethylaminoazobenzene-1-azo-2-naphthalene, or 3'-p-dimethylaminoazobenzene. Some male and female rats were gonadectomized and pellets containing estrogen and testosterone respectively were implanted in some of them. Some females were adrenalectomized unilaterally.

B. A sarcoma induced in Osborne-Mendel rats in this laboratory was transplanted in male animals of the same strain. Another group of male and female Osborne-Mendel rats was fed on semisynthetic diet containing p-dimethylaminoazobenzene-1-azo-2-naphthalene. When tumors were palpable and reached a size of about 4 cm. in diameter, tissue and serum aldolase was determined on normal and tumor-bearing rats.

Major Findings: A. Female rats had lower incidence of cancer than males. A paper based on these findings has been published. Studies on gonadectomized, adrenalectomized, and hormone treated rats are still in progress and no findings are yet available on them.

Part A (continued)

B. Serum aldolase values of sarcoma and hepatoma bearing rats were respectively 3 and 6 times the values for normal rats without tumors. Aldolase levels for liver tissue of tumor-bearing rats were about 30% lower than those for the liver tissue of control rats. A paper based on these findings has been published.

Significance: These studies help to define environmental conditions which influence the growth of these tumors and the changes tumors induce in their environment.

Projected Course: The differences between male and female animal, which might be responsible for differences in tumor growth between the sexes are currently under study.

Part B included: Yes

NIH-NCI
Individual Project Report
Calendar Year 1959

Part 3: Honors, Awards, and Publications

1. Mulay, A. S., and O'Gara, R. W.:
Incidence of liver tumors in male and female rats fed carcinogenic azo dyes.
Proc. Soc. Exp. Biol. & Med. 100: 320-322, 1959.

2. Mulay, A. S.
Tissue and serum aldolase of rats with primary hepatoma.
Proc. Soc. Exp. Biol. & Med. 101: 763-766, 1959.

2. Cancer Endoc. & Ex
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of the Growth of Adrenocortical Carcinoma on the Chemistry and Physiology of the Host Rat.

Principal Investigator: Ambadas S. Mulay

Other Investigator: Edward B. Price

Cooperating Units:

Man Years (calendar year 1959):
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To characterize the steroids secreted by this transplantable host rat as a biological test animal.

Methods: Transplanting the adrenocortical carcinoma in male and female rats, intact and adrenalectomized. Running potassium tolerance tests and glycogen deposition tests on adrenalectomized rats with and without tumors. Sodium and potassium studies on rats with and without tumors, intact and adrenalectomized. Other clinical chemistry studies on these animals.

Major Findings: Potassium tolerance tests show that this tumor secretes mineralocorticoids. A paper based on these findings has been submitted for publication. Results of clinical chemistry and physiology study were reported last year. Sodium and potassium study indicate mineralocorticoid secretion by the tumor. A paper based on these findings is in preparation. Preliminary results on glycogen deposition tests indicate that this tumor secretes little, if any, glucocorticoids.

Significance: These findings in part, define the steroidal characteristics of this tumor. Failure to synthesize certain steroids by this tumor invites an inquiry about factors responsible for this failure.

Proposed Course: Tests for glucocorticoid secretion by this tumor are still under study. Metabolism experiments are being planned to investigate factors which block synthesis of certain steroids by the tumor tissue.

Part B included: No.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Induction of Multiple Tumors in [illegible] Mammals with Stewart-Eddy Polyoma Virus-tissue Culture Preparations

Principal Investigator: Mearl F. Stanton

Other Investigators: Robert Blackwell, Sarah Stewart, Bernice Eddy [illegible] Joseph Albrecht and staff

Cooperating Units: DBS, Laboratory of Biology, NCI

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: This is a continuation of projects outlined in Individual Project Report NCI 516, 1957.

Major Findings: Summaries of major findings in cooperation with other investigators will be found in their reports. In addition, separate investigations of host factors involved in induction by this laboratory have established the following: (1) In collaboration with Drs. Kahler and Stewart an analysis of tumor incidence has been made in mice inoculated by limiting dilutions of the virus culture preparations that were subjected to differential high speed centrifugation. All fractions induced tumors although most of the activity could be sedimented at a particle size consistent with that of the virus. The variety of tumors did not vary significantly in the different fractions and no significant incidence of leukemia was observed. The limiting dilution and fractionation allowed an analysis of tumors that follow minimal doses after long-term study. Many non-specific lesions particularly in the kidneys and salivary glands of older mice indicated either abortive attempts at cell proliferation or regression of minimal lesions. Older mice also had a significant number of tumors particularly bone tumors that showed clear evidence of acquiring characteristics of invasion and metastases.

(2) The transplantable hamster tumors referred to in NCI [illegible] have continued in transplant. Serological attempts to detect antibody in grafted hamsters have been made on a limited basis. Thus far significant titer antibody has not been detected in the grafted animals. Present studies are being carried out on grafted newborn hamsters. Much of the work with grafted tumors is directed toward determining what influence host factors may have on the behavior of grafted tumors and induced tumors. Two systems are used: (a) varying host factors (age, sex, carcinogen exposure, [illegible] exposure, etc.) in animals grafted with tumor lines of known behavior, (b) inducing tumors of varying type and behavior from single tissues [illegible]

Part A (continued).

to virus-cultures and implanted into the cheek pouch of weanling hamsters.

(3) A critical analysis of the tumors in mice has been published (Reference 1). Original observations in this study include conclusive support for theory that the active agent is a virus and relation of tumor development to availability of passively transferred antibody.

Significance: The polyoma virus-tumors serve as exceptional experimental tools. The wide range of species that respond and convenience in working with small rodents is an advantage over all other virus tumors. Secondly there is the advantage over chemically induced tumors both in the short latent period and opportunity of following the progress and behavior of the tumors in relation to virus as detected by new virological and morphological techniques including fluorescence antibody techniques. Much of our more recent activities center on perfecting the fluorescence technique. Finally there is good evidence to indicate that induction and progression proceed in a slow step-wise fashion which may allow separate study of the two most important attributes of tumors namely cell proliferation and the acquisition of agressive behavior patterns by the proliferating cells.

Proposed Course: The development of a simple basic system for tissue culture preparation enabling this laboratory to carry out experiments on host factors with a single standard line of virus culture preparations. Development of fluorescent antibody techniques for the detection of polyoma virus antigen. Preparation of a report on the histogenesis of tumors which develop in the hamster.

Part B. included: Yes

[illegible]. Lectures, Awards and Publications

Publications other than abstracts from this project:

1. Stanton, M. F., Stewart, S. E., Eddy, B. E., and Blackwell, V.
The oncogenic effect of tissue culture preparations of polyoma virus on fetal mice.
J. Nat. Cancer Institute. December 1959.

2. Stanton, M. F.
The local oncogenic response of hamster tissues to polyoma virus.
Cancer Research. In press.

3. Stewart, S. E., Eddy, B. E., and Stanton, M. F.
Neoplasms in rodents induced by S. E. Polyoma virus.
Acta Unio Internationale Contre le Cancer XV: 842-851, 1959.

NCI 516
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental Induction of Adenocarcinoma of the Glandular Stomach in the Mouse

Principal Investigator: Edward B. Price, Jr.

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2
Other:

Project Description:

Objectives: The experimental induction of adenocarcinoma of the glandular stomach of C57BL mice by intramural injection of methylcholanthrene has been reported by Stewart and Lorenz in 1942 and by Hare et al in 1951. However, the overall yield of tumors produced by this method was small. It was decided to attempt the same procedure in cortisonized mice to determine if the yield of tumors obtained would be greater than that with methylcholanthrene alone.

Methods: Routine

Major Findings: The project is not as yet completed, but the findings to date suggest that cortisone has not enhanced the yield of methylcholanthrene induced tumors of the glandular stomach in mice.

Significance: Studies of the genesis of carcinoma of the stomach would be greatly enhanced if a method could be devised for the experimental induction of that lesion.

Proposed Course: This study has been delayed by the completion of the collaborative projects listed below. It is proposed to complete the project.

Part A (continued)

Other projects in collaboration with others:

1. Transplantable thiouracil-induced thyroid tumors in the rat with Dr. Seymour Wollman. Report submitted to Dr. Wollman on October 15, 1959.

2. Transplantable adrenal cortical carcinoma in the rat with Dr. A. S. Mulay. Report submitted to Dr. Mulay on July 15, 1959.

3. Pathologic findings in the human kidney in patients with acute leukemia with Drs. E. Frei and R. Fritz. Report submitted to Dr. Frei on June 15, 1959.

4. The fate of intravenously injected melanoma cells in the lung and the relationship to the effectiveness of chemotherapy: An experimental study with Drs. D. L. Kinsey and R. [illegible]. Report submitted to Dr. Kinsey May 30, 1959.

Part B. included: Yes

Part II. Honors, Awards, and Publications

Publications other than abstracts from this project:

Kinsey, D. L., Price, Edward B., Jr., and Smith, Robert R.
Effectiveness of Chemotherapy in Relation to Extravascular Invasion by Embolic Tumor Cells.
Accepted for publication by CANCER.

Serial No. [illegible]
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Md.

PHS-NIH
Intramural Project Report
Calendar Year 1959

Part A.

Project Title: Corticosteroid Metabolism of an Adrenocortical Adenocarcinoma of Rat.

Principal Investigator: Amadeo S. Muley

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To extract, purify, isolate, and identify, corticosteroids secreted by the tumor and those excreted in the urine of tumor-bearing rats.

Methods: Growing the adenocarcinoma in rats, and harvesting and preserving the tumor tissue. Collecting urines from tumor-bearing rats, using metabolism cages. Extraction of homogenized tumor tissue with ethanol and separating the extract from solid residue. Separation of lipids from the extract by freezing and salting out techniques. Evaporation of ethanol in vacuo at low temperature and extraction of the aqueous residue with ethyl acetate, purification of the extract and removing ethyl acetate in vacuo. Steroid residue thus prepared, column chromatographed into 5 fractions on an aluminum silicate column. The corticosteroid fractions were chromatographed on paper with various solvent systems, paper strips developed to locate steroid spots, the spots eluted and rechromatographed with different solvent systems. Steroid spots on the chromatographs were visualized in ultraviolet light and with various chemical agents.

Major Findings: Preliminary results show indications of steroids close to etiocholanolone, androstanedione, progesterone, testosterone, and a large spot of highly polar steroid, more polar than Reichstein's E- the most polar standard used.

Part A (continued)

Significance: Identification of the steroids secreted by this tumor may show us as to where the steroid metabolism of the tumor differs from that of the normal adrenal gland. This in turn may lead to factors that influence these changes.

Proposed Course: Extraction of more tumor and fractionation of the steroids, to collect enough material in each fraction, to characterize and identify the steroid compound.

Part B included: No

Serial No. NCI 220
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part I.

Project Title: Morphologic Study of the Effects of Radiation Protective Compounds

Principal Investigators: Roger W. O'Gara and Margaret G. Kelly

Other Investigator: Joanne Hollcroft

Cooperating Units: General Medicine Branch, NCI
Radiation Branch, NCI

Man Years (calendar year 1959):
Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives: To determine, if possible, the mode of action of radiation protective substances on the toxic changes produced by HN_2 and other radiomimetic chemicals.

Methods: Autoradiographs of tissue from mice given S^{35} and C^{14} labeled AET were used to estimate tissue distribution of AET.

Major Findings: 1) Autoradiographs of tissue blocks show localization of S^{35} AET in liver, gut, bone marrow, red pulp of the spleen, and in the proximal convoluted tubules and papillae of the kidney. Concentration of S^{35} AET is low in lymphoid tissue (lymph nodes, thymus gland and follicles of the spleen). It is also low in skeletal muscle, transplanted tumors (primary and metastatic), skin, and brain. 2) Only a few satisfactory microautoradiographs have been obtained to date, but they appear to substantiate the observations listed above and suggest that the intracellular distribution is chiefly to the cytoplasm. In the red pulp of the spleen, the labeled AET appears to be extracellular rather than associated with any particular cell. The brain contains very little radioactivity, but what is there appears to be concentrated about the larger neurons. 3) Autoradiographs of tissue blocks from mice given C^{14}-labeled AET gave an identical picture to that obtained with S^{35}-labeled AET. In contrast, autoradiographs of normal and tumor-bearing mice killed 30 minutes after administration of S^{35} and C^{14} labeled thiourea and S^{35} labeled sodium sulfate show a low but uniform distribution of radioactivity throughout the body tissues.

[illegible]
Individual Project Report
Calendar Year 1959

Part A (continued)

Significance: It may help explain the variation in the effectiveness of AMT in different species of animals, e.g., between the dog and the mouse.

Proposed Course: 1) To study the morphologic actions of other radiation protective substances as they become available. 2) To study the comparative tissue distribution in dogs and rodents.

Part B included: Yes

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Kelly, Margaret G., Hall, David P., Trivers, Glenn E., O'Gara, Roger W., and Eubrod, Charles G.
Actions of S,2-aminoethylisothiuronium Br. HBR (AET). Toxicity and Protective Effect Against Nitrogen Mustard Toxicity.
J. Pharmacology and Experimental Therapeutics. In press.

[illegible]
Laboratory Project Report
Calendar Year 1959

Part I

Project Title: Morphologic and Biologic Studies of Leukemias and Lymphomas of the Mouse in Tissue Culture

Principal Investigator: Clyde J. Dunn

Other Investigators: Michael Potter

Cooperating Units:

Man Years (estimate year 1960):
Total: 4 5/6
Professional: 1
Other: 3/6

Project Description:

Objective: To observe the morphologic changes of [illegible] cells of the mouse in tissue culture, and to correlate these changes with alterations in neoplastic characteristics.

Methods: Continuous cultures of leukemias or lymphomas are established by methods described in previous reports (1957, 1958). The cytologic characteristics in culture and in transplanted hosts are compared as well as the biologic differences between the culture line and [illegible] transplantable mouse tumors. These problems that have been studied are described below.

Major Findings: A. Reticulum cell sarcoma P329. Little advance in terms of positive findings has been made with this line [illegible] thereby described in the 1958 report. Several sublines that had been [illegible] for as long as two years have lost through accident, interrupting the study on effect of alternate passage through cultures and mice. The original culture line has been maintained, however, and several [illegible] derived from the host culture lines have been carried in mouse passage [illegible] lines from tumors that grew in mice inoculated as newborns or after x-irradiation, with low-virulence cells) are [illegible] maintained in mouse passage.

Attempts to transfer "virulence" to the avirulent line have been unsuccessful. Two approaches were used, as follows: (1) Filtrates and crude extracts from short-term cultures of the virulent [illegible] P329 line were added to cultures of the avirulent li[illegible]

Part A (continued)

these cultures were injected IP into compatible mice. No tumors appeared. (2) A spontaneous histiocytic neoplasm very similar to the original P329 neoplasm was encountered in an old BALB/c x DBA/2 mouse. Since P329 had arisen in a DBA/2 mouse this situation provided us with a virulent, cytologically identical tumor, differing from P329 in its F_1 genetic constitution (and associated histo-compatibility characters) as well as in virulence. To test the possibility that a "virulence factor" might be transferred directly from cell to cell by contact, mixed population cultures were prepared by adding cells of the virulent F_1 tumor to cultures of the avirulent P329. After a week of contact in these mixed cultures, the mixed populations were injected IP into DBA/2 and /BALB/c x DBA/2/F_1 hybrids. Tumors grew in and killed the F_1 hosts, but no tumors appeared in the DBA/2 hosts, indicating that a "virulence factor" either had not been transferred or that if it had been transferred, the F_1 histo-compatibility antigens were linked to it, preventing transplantation to the parent strain. From this it could also be concluded that if an "avirulence factor" had been transferred, it had not been transferred to all of the virulent cells.

B. P388. Cells from this lymphoid neoplasm have been carried in culture for over three years. Three separate isolations are being carried and each of these lines shows a characteristic cell morphology, although the similarities within the group are greater than the differences. All of these lines show marked morphologic and biologic alterations that were described in last year's report. In addition, studies by time-lapse cinematography are being made. These show that the manner of locomotion of the altered tissue culture cells is not similar to that of lymphoid cells, but, like their cytologic features in fixed preparations, is more similar to movement of macrophages in culture.

Dr. Robert Noone of the Leukemia Study Section has used $P388D_1$ cells to study development of drug resistance in vitro. He has submitted tumors, derived from cultures resistant to several anti-leukemic drugs, for histologic study. At present it appears that tumors derived from cells resistant to 5-fluorouridine can be distinguished from tumors derived from other $P388D_1$ culture sublines. This point needs further study to establish more objectively what the special features of the 5-fluorouridine resistant cells are in terms of physical or histochemical qualities.

C. P815. Active studies of this culture cell line, derived from mast cell tumor P815, have been discontinued, although a few cultures are still maintained. The cultures no longer contain any cells identifiable as derivatives of mast cells, and cultures inoculated into mice fail to produce tumors. The cells that continue in culture resemble fibroblasts.

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Part A (continued)

Significance: The change in morphology and the complete or partial loss of virulence of the above neoplasms during propagation in vitro seems likely to be of fundamental importance, perhaps comparable to the loss of virulence of pathogenic bacteria and viruses when cultivated in vitro. The phenomenon may well be similar to that originally described by Sanford for high and low tumor-producing lines developing from normal mouse fibroblasts in vitro. It perhaps has somewhat different meaning than the phenomenon observed in Sanford's system, inasmuch as in our experiments the neoplastic cells had already possessed tumor-producing qualities before being placed in culture. If the mechanism by which loss of virulence occurs could be determined, it is conceivable (remotely) that the change could be induced in vivo, thus increasing survival of tumor-bearing animals. On the other hand, in the analogous bacterial systems, methods have never been found for decreasing virulence in vivo, though transfer of DNA has been shown to confer increased virulence on previously avirulent strains in vitro.

Proposed Course: The negative results in all attempts to restore virulence to P339 cells have frankly been discouraging, although all the avenues of approach have not been closed. For example: (1) transfer of purified DNA extracts from virulent cells to avirulent ones has not been attempted. (2) Transfer of avirulent cells within millipore chambers, followed by release of the cells into the same host animal might provide advantages for successful transplantation. (3) Transduction of a theoretical virulence factor by means of a virus could be attempted, particularly as the P339 line has been shown to be susceptible to infection by polyoma virus. However, experiments along these lines have been and still are being deferred in order to pursue intensively other projects concerning intercellular relationships in tissues infected with polyoma virus in vitro.

Part B included: No

1. Laboratory of Pathology
2. Cancer Indust. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Tissue Culture Studies Investigating the Mechanism of Tumor Induction by Parotid Tumor Agent (Polyoma Virus)

Principal Investigator: Clyde J. Dawe

Other Investigators: L. W. Law

Cooperating Units:

Man Years (calendar year 1959):
Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives: This project is an extension of Project 3Ila described in the 1958 report. The investigation of parotid tumor agent in leukemic tissues of mice has been published, and the observations on changes induced by the agent on newborn mouse salivary glands in vitro are in press. The objectives of this project are to determine the influence of several host factors on the development of the two basic types of response to the virus in vitro; namely, the cytolytic and the proliferative responses. The host factors presently being investigated are: chronologic age, species differences, immunologic state with respect to polyoma virus infection, stage of cellular differentiation at time of infection, and specific influence of mesenchymal elements on the development of the different types of response by epithelium.

Methods: The investigation employs both the gelatin sponge matrix method of tissue culture described in publication (2) below, and the salivary gland rudiment culture technique first described by Borghare and later modified by Grobstein. Submaxillary gland tissue or submaxillary rudiments at various stages of development are cultivated on plasma clot adjacent to gelatin sponge. In the case of adult submaxillary gland, it is necessary to replace the sponges after 7-10 days of culture because during that period adult glands secrete a protease that digests the original gelatin sponge.

Part A (continued)

To study influences of state of cellular differentiation and of morphogenetic evocators on the type of response to virus, submaxillary gland rudiments are cultured with and without virus, at various stages of development ranging from the earliest single-bud anlage to stages of adenomere formation and functional acinar development. To study the effects of virus on the epithelial or mesenchymal rudiments alone, these components are first separated from one another by the trypinization technique of Grobstein. Coupled with recombination experiments, this approach makes possible the analysis of intercellular relationships and their influence on the result of viral infection.

Major Findings: Increasing chronologic age beyond the newborn period does not diminish the proliferative response of submaxillary gland to parotid tumor agent in vitro. Precise quantitation in this system is difficult, but estimations are that the proliferative response is more pronounced in adult glands than in glands of newborns, in vitro. Certain qualitative differences have been noted also, which will not be described here. No significant difference has been noted between adult male and adult female response. Glands from mice at 4, 6, and 20 weeks and at 13 months of age give approximately similar response.

In the opposite direction our observations are incomplete, but indicate thus far that there is little or no proliferative response by the epithelial elements in glands in the earliest stages of morphogenesis. Cells of the epithelial as well as of the mesenchymal portions of the rudiments are infected, however, as indicated by typical nuclear lesions and necrosis. The cytolytic phenomenon appears to be about as marked in the rudimental mesenchyme as in the adult mesenchyme, but the epithelial response is predominantly, if not entirely, cytolytic in the embryonic rudiments, whereas it is chiefly proliferative in epithelium of adult glands in vitro. Results with separated epithelial and mesenchymal components of rudiments are not yet at hand.

A single experiment testing the response of submaxillary gland from a 15-month-old mouse, "naturally" infected by parotid tumor agent and having a high HI serum titer, has been carried out. The proliferative response at 30 days was just as marked as in adult gland from non-immunized mice. Further, the uninfected control cultures showed no response to virus at all, indicating that latent virus was not present or not effective in the usual manner, even though added virus was able to act.

Studies of differences of species response have not been completed. It has been found, however, that parotid, submaxillary, thyroid, and thymus gland tissue from two human fetuses did not respond in either a cytolytic or proliferative manner. Studies with rat and hamster salivary glands are under way.

Part A (continued)

It is important to the interpretation of the above findings to ascertain the biologic meaning of the morphologic changes observed in cultures infected with the virus. Initial attempts to transplant the altered cultures to mice resulted in development of virus-induced tumors in the X-irradiated adult recipients, but not in the growth of tumors from the transplanted cells.

Renewed attempts to graft the cultures are under way. Glands from adult mice have been removed surgically, infected and cultured for 30 days, and then returned to the original donors, as well as to isologous hosts. In some cases they have been placed under the capsule of the remaining submaxillary gland. Cultures have been transplanted to immunized and non-immunized adults, as well as to newborn mice. Growth of tumors at the sites of transplant has not yet been observed after periods ranging upward from six weeks.

Significance: Because of the failure of altered cultures to grow into tumors when transferred to mice, the changes in vitro cannot at present be accepted as fully equivalent to tumor genesis in vivo. However, it must also be recognized that the transplantability characteristics of comparably small and early tumors induced in vivo is not known. It is known that even large salivary gland tumors succeed in becoming established in new hosts in much less than 100% of the attempts. Virus-induced thymic tumors grow only rarely on transplantation. It is our tentative assumption that the proliferative and morphologic changes in cultures are in fact equivalent to the early changes in vivo at a stage where continued growth is still highly dependent on host influences.

If this assumption can be verified then it is apparent that the findings described above offer a challenge to the concept that either early chronologic age (meaning ?) or relatively undifferentiated state favor tumor induction by this agent. On the contrary, it appears that the newborn mouse is peculiarly susceptible to the tumor-inducing effects of the agent because it is precisely at this age that two necessary conditions overlap in time; namely, (1) susceptibility to infection by virtue of incompletely developed immune response, and (2) capability for proliferative response by virtue of relatively, though not completely advanced state of morphogenesis and/or differentiation. This conclusion is supported by our previous observations that the apparent refractoriness of adult animals could be broken down by (1) introduction of a massive dose of virus by IV route; and (2) by impairing the immune response by irradiation prior to virus inoculation.

Part A (continued)

There is a practical value in these observations with regard to search for viral tumor agents in humans. If analogous agents exist in humans, it would appear that cultures of adult tissue would provide more favorable test systems than cultures of newborn or fetal tissues. Also, the state of immunity of the donor of the tissue to be infected in culture could be disregarded, as this would not affect the response (assuming use of non-immune sera from heterologous sources in the culture medium). These two considerations should facilitate the use of this system as a model for detection of tumor-inducing viruses.

Two hypotheses as to the intercellular and cell-virus relationship concerned in tumor induction by this virus are under consideration as follows: 1) Three-factor, or double dependence theory. The proliferative response of salivary gland epithelium to infection by the agent is dependent on the prior establishment of a certain level of differentiation in the infected cell. This level of differentiation in turn is dependent (as shown by embryologic studies) upon specific interrelationships between the epithelial cells and the supporting mesenchyme. If this interrelationship has not been achieved (in embryogenesis) or has been interrupted (separation of epithelium from stroma in preparation of monolayer cultures), the proliferative response is supplanted by a cytolytic one. The specificity of susceptibility and of response of certain organs and cells to the agent may thus be based on a competitive relationship between cell and virus for the complex materials (RNP ?) by which the mesenchymal supporting cells normally control differentiation and function.

According to this concept, a third element, the mesenchymal supporting cell, must be taken into consideration in the study of virus-cell relationships in tumor genesis. Tumor development would be dependent not only on the presence and action of virus in the tumor cells, but also on the maintenance, at least for a period during the evolution of the tumor, of the dependent relationship of the infected cell with a properly functioning mesenchymal supporting cell. This concept if double dependence would give the mesenchymal cell the role of maintaining the "balance" between virus and host cell which many virologists have believed essential for development of virus tumors. When the supportive cell is removed from the picture, the infected cell is no longer able to compete and the virus multiplies and destroys the cell.

This concept is consistent with the observation of unsuccessful transplantation of tissue cultures and tumors. We have noted that the mesenchymal cells in our tissue cultures had undergone very nearly complete necrosis at the time transplantation was attempted. Hence, it would follow

Part A (continued)

that the epithelial cells, though started along a proliferative pathway would eventually succumb to viral effects because the system had been deprived of the third influence. This would also explain the failure of growth of the showers of embolic pulmonary metastases that occur from the salivary tumors.

The above concept could account for a favorable influence of viral antibody on tumor growth, once initiated. As the mesenchymal cells have been shown to be lethally responsive to virus infection, their protection by some means would be necessary to maintain them in their supportive function. Here again, the time of development of antibody response in the mouse happens to be ideal for provision of this protection to the mesenchyme.

2) Two-factor, dose-response therapy. A reconstruction of the chain of events according to this concept runs as follows: The first step in viral infection involves the mesenchymal cells in contact with epithelium. In the absence of antibody, virus multiplies in these cells and eventually produces cytolysis, with local release of immensely increased, concentrations of virus. In the adult gland, this high virus concentration infects the differentiated epithelium, resulting in a proliferative reaction, whereas in poorly differentiated embryonic epithelium it produces only cytolysis, because the undifferentiated epithelium is more "sensitive." If this concept were correct, it should be possible to induce proliferative effects in embryonic epithelium by using a lower virus dose. This is difficult to achieve in a complete organ, since virus boosts its concentration by replicating in the mesenchymal cells. Virus dose could be controlled only in a pure epithelial system.

It will be noted that even this concept requires some explanation for the difference in reaction of embryonic epithelium as compared with adult epithelium, assuming that the dose level to which they are exposed in organ cultures is the same. This theory is antithetic to the first in that its premise is that less well-differentiated epithelium is more susceptible to either the cytolytic effect or the proliferative effect of virus, the type of response depending only on the initial dose. The first theory holds that embryonic and adult epithelium respond differently to the same dose of virus because of influences of a third factor, the controlling mesenchyme.

Proposed Course: The projects below have been selected for future investigations chiefly on the basis that they will be of value in determining the validity of one or the other of the above concepts of tumor genesis.

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Part A (continued)

1. The studies of differences of species response in vitro will be continued at least until the hamster and rat have been included. Extension to other animals will depend on results with these two. We are especially interested in obtaining information as to whether a proliferative response in a given species correlates with the ability of the salivary gland mesenchyme of that species to promote morphogenesis and differentiation of salivary gland epithelium of the mouse.

2. Investigation will be continued on the effect in vitro of the virus on the separated submaxillary rudiments of the mouse. If the first concept is correct, the virus should produce either necrosis or no effect at all on either isolated capsular mesenchyme or isolated epithelial rudiment. If proliferation occurs in isolated epithelial rudiment, the first concept will have lost much of its support.

3. It is hoped to be able to study the effect of viral antibody on the proliferative phenomenon in vitro. This can be done by repeating the experiment as originally carried out, but adding antibody to the medium at various time intervals after infection. This experiment will require removal of anti-mouse antibody from the antibody preparation.

4. Continued attempts will be made to find a way to achieve transplantation of the converted cultures to mice. Possibly the addition of antibody to cultures after the initiation of the morphologic change but prior to transplantation would be helpful. Another possible tactic would be to grow the cultures in millipore chambers in the mouse before removing them and placing in contact with host tissue. A successful result could be interpreted to indicate an influence of isologous serum environment during the conversion process.

5. As a double check on the presently-held view that a relatively advanced state of differentiation of the epithelium actually favors the proliferative type of response, cultures not exposed to virus will be carried for 30 days or more, after which time virus will be added and the results observed. Although the epithelium survives well for 30 days, by this time is has lost much of its differentiated character, and theoretically should give a less pronounced proliferative response than fully differentiated adult glands.

Part B included: Yes

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Dawe, C. J., Law, L. W., and Dunn, T. B.
Studies of parotid-tumor agent in cultures of leukemic tissues of mice.
J. Nat. Cancer Inst. 23: 717-797, 1959.

2. Dawe, C. J., and Law, L. W.
Morphologic changes in salivary-gland tissue of the newborn mouse exposed to parotid-tumor agent in vitro.
J. Nat. Cancer Inst. 23: 1157-1177, 1959.

3. Banfield, W. G., Dawe, C. J., and Brindley, Darlene C.
Intracellular and extracellular particles in tissue culture inoculated with parotid-tumor agent (polyoma virus).
J. Nat. Cancer Inst. 23: 1123-1135, 1959.

4. Law, L. W., Dawe, C. J., Rowe, W. P., and Hartley, J. W.
Antibody status of mice and response of their litters to parotid-tumor virus (polyoma virus).
Nature, 184: 1420, 1959.

1. Laboratory of Animal
1. Cancer Induc. & Pathogenesis
1. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Induction and Natural History of Conjunctival Carcinoma in Hereford Cattle

Principal Investigator: Maarl F. Stanton

Other Investigators: Robert Zeigel, Robert Manaker, Ray Bryan, W. I. Gay,

Cooperating Units: Laboratory Aids Branch, Division of Research Services, Laboratory of Biology, NCI

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: To determine the fate of certain proliferative epithelial lesions on the eyes of cattle and to attempt by artificial means to induce carcinoma in cattle with the products of the tumors which occur naturally.

Methods: Viable tumor tissue and cell free extracts of conjunctival carcinomas of Hereford cattle, prepared by concentration techniques successfully established for Rous sarcoma virus, have been inoculated into the conjunctivae and udders of Hereford cattle. Both young unaffected animals and animals with conjunctival carcinoma have been used as recipients of the inocula. Dr. R. Zeigel does electron microscopy on biopsied tumors.

Major Findings: A herd of 500 cattle in Eastern Maryland has been studied. Ten to 20% of the herd has eye tumors. Systematic follow up of progressing lesions with photographs is in progress. Biopsied material has been used for electron microscopy and tissue culture study as well as inoculation. The conjunctive of 4 cattle have been inoculated with tumor extracts or culture material. Thus far we have uncovered no evidence other than epidemiological evidence that this tumor is etiologically related to micro-organisms. However, further work is in progress through morphological studies by Dr. Zeigel and tissue culture studies by Dr. Manaker.

Significance: This is an unquestionably malignant epithelial tumor which has its counterpart in man, and follows the natural history of human epidermoid carcinoma. The high incidence of such a lesion in a herd of cattle suggests a common etiological factor not as yet apparent. A most

Part A (continued)

likely possibility is that the initial verrucous lesion is induced by insect-borne viruses. Subsequent progression to malignancy may be a part of or separate from the initiating process. The cow serves as an excellent animal in which to study this problem, of added significance is the possibility of uncovering means of preventing the disease in cattle, a serious economic hazard in present day animal husbandry.

Proposed Course: It seems likely that the precursor to the malignant lesion is the hyperkeratotic verrucous wart noted in many of the Virginia cattle. We shall attempt to collect, recover, and induce typical lesions with such material as well as with concentrated products of the malignant lesion.

Part B included: No.

Serial No. NCI 522
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effects of Ionising Radiation with Emphasis on Morphologic Alterations and Neoplasms

Principal Investigator: Richard L. Swarm

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: The collection of information regarding the changes effected by ionising radiation of different types in man and experimental animals.

Methods: 1) Detection and study of neoplasms and other morphologic changes in experimental animals who have received external radiation or internally administered radioactive isotopes.

2) A study of the distribution, metabolism and effects of radioactive isotopes of several types in man and animals. Here the emphasis is on the utilization of gross and histologic autoradiographic techniques in the study of thorium dioxide, S^{35} and I^{131} labeled materials.

3) Participation in the activities of the Pathologic Anatomy Department. Special emphasis is made in the study of human specimens demonstrating change due to irradiation or containing radioactive isotopes.

4) To facilitate the above and to assist other investigators, collaboration on problems in this field is fostered. Studies on the distribution of S^{35} in human tissues have been in collaboration with clinical studies of Dr. J. Robert Andrews and others in the Radiation Branch, NCI.

Major Findings: From the continuing study of the reported effects of colloidal thorium dioxide administered intravenously in man, the picture of focal scarring of the liver emerges as a lesion which at times is described in association with neoplasms, but is more frequently the sole pathologic lesion in man. Most of the animals and certain of the human

Part A (continued)

patients with malignant vascular tumors following intravenous colloidal dioxide have little or no focal scarring in the liver or spleen although scarring is seen in regional nodes and subcutaneous tissue containing thorium dioxide. In experimental animals scarring of the subcutaneous tissues about extravasated thorium deposits does not appear to be as extensive as the scarring in man. On the other hand, tumors at the site of subcutaneous extravasation of thorotrast are more common in rodents than human patients. In view of these findings further study of the correlation of fibrosis and neoplasia in the liver and spleen of human patients with thorotrast deposits is planned.

Work with the transplantable vascular tumors of the liver and spleen in mice has continued. Serial transplantation has resulted in some increase in the growth rate and morphologic changes. Preliminary morphologic studies indicate that the slower growth rate is associated with well differentiated tumors which have small vascular structures. Faster growing tumors have cells present in more of an aggregate with less well defined vascular structures. A careful study of the hematologic system is in progress in these animals with vascular tumors.

Study of rabbits with intravenously injected colloidal thorium dioxide has continued. During the past year two more animals have died with liver and spleen hemangioendotheliomas. These animals showed no fibrosis. The number of rabbits on experiment have been small but the following data shows a definite trend. In 12 animals injected with thorotrast (lot 120) 3 animals received 2 injections of 3 cc. and 9 animals received a single injection of 3 cc. Of the 3 animals which received a total of 6 cc. one died without tumor after 19 months. The other two both developed tumor at 36-37 months. None of the animals given a single injection of 3 cc. developed tumor; however, only 4 of the 9 in this group lived longer than the 38 months. (Six in the group died without tumor within 35 months after injection.) Of the 4 animals which lived 38 months or longer only one which is yet alive has lived 43 months.

Thorotrast given intravenously apparently tends to increase the number of deaths from infection, etc. While this has not been an object of direct study, it has plagued our experiments on carcinogenesis, particularly those in rats. An experiment started in OM/N rats with Dr. Rabe to study the effects of partial hepatectomy and splenectomy on the incidence of neoplasia has been virtually reduced to a pilot experiment by numerous deaths primarily from pulmonary infection. Similar but less extensive difficulties were experienced with experiments with mice and rabbits.

One patient with thorotrast (02-14-58) was studied in the Clinical Center during the past year.

Part A (continued)

Significance: With increased exposure of man to radiation, it has become more and more important to establish the pathogenesis and character of irradiation injury and irradiation induced neoplasia so that injury leading to neoplasia can be prevented if possible and so that radiant energy can be used in the most satisfactory manner to destroy neoplasms.

Proposed Course: 1) The activities outlined under Methods are to be continued.

2) In an effort to reduce the latent period for neoplasia to develop, the administration of thorotrast to newborn rabbits and the use of litter mate controls receiving thorotrast at a later age is planned.

3) Close attention will be paid to the reports of others studying human patients with intravenously administered thorotrast.

Part B included: No

Serial No. NCI 541

1. Laboratory of Pathology
2. Cancer Induc.& Pathogenes
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pathologic Findings in Human Trophoblastic Disease.

Principal Investigator: Edward D. Price, Jr.

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2
Other:

Project Description:

Objectives: Despite the voluminous literature on the morphologic aspects of choriocarcinoma and related conditions, reports of well-studied series of large numbers of cases are rare. It is felt that the availability of a relatively large number of such cases, particularly an ample series of necropsies of choriocarcinoma, at the Clinical Center provides suitable material for a careful morphologic study of this group of conditions.

Methods: Routine

Major Findings: The project is still in the planning stage and there are no significant findings as yet.

Significance: The study may help to clarify some of the confusion which still exists about the morphologic aspects of this group of conditions.

Proposed Course: Study of the clinical records, autopsy reports, and pathologic material available.

Part B included: No.

Serial No. NCI 52c
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemical Carcinogenesis

Principal Investigator: Marie L. Hesselbach

Other Investigator: Roger W. O'Gara

Cooperating Units: NIAID

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: 1) To study the morphology of light green and fast green induced tumors. 2) To study the changes in mitochondria and in the intracellular glycolytic and respiratory enzyme systems induced by these dyes.

Methods: Rats were injected subcutaneously 2-3 times weekly with fast green and light green dyes (3% and 6% aqueous solutions).

Major Findings: 1) Subcutaneous fibrosarcomas were induced in over 90% of animals by both dyes when injected as a 3% or 6% solution in distilled water. The first tumor arose 7 months after the start of injections in a fast green treated animal. The tumors spread readily by metastases, implantation, and direct invasion and grew consistently on transplantation. 2) The adrenal glands and gonads showed marked hyperemia and hypertrophy in animals bearing transplants of one subline of tumors but not in animals bearing implants of the other subline. These lesions are similar to those that have been reported in animals with hypervolemia.

Significance: Should supply fundamental knowledge on the relationship between mitochondria and neoplasia.

Proposed Course: No further work is planned at the present time.

Part B included: Yes

Part E. Honors, Awards, and Publications

Publications other than abstracts from this project:

Rasselbach, M. L., and O'Gara, R. W.
Feet Green- and Light Green-induced Tumors: Induction, Morphology, and Effect on Host.
J. Nat. Cancer Inst. In press.

1. Laboratory of Experimental
2. Cancer Cause, & Prevention
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemical Carcinogenesis

Principal Investigator: Roger W. O'Gara

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: To study several popular cathartic compounds for possible carcinogenic properties.

Methods: Using three different strains of mice, six different cathartics were administered either in the diet or by stomach tube for 15-20 months.

Major Findings: In RAIB/c male mice living to one year or older 9 of 25 control mice had lung tumors, one had a squamous cell carcinoma of the submaxillary gland, one had an interstitial cell tumor of the testis and one showed reticular cell hyperplasia of abdominal lymph nodes. Mice receiving cascara, rhubarb, castor oil, or phenophthalein respectively in their food (powdered Donwood pellets) had essentially the same incidence of tumors as the control animals, although there were minor variations. Three of the 12 animals receiving podophyllum in their diet developed hepatomas, eight showed either lymphoid tumors or marked reticular cell hyperplasia of liver, spleen and lymph nodes, two had benign polyps of the cecum or colon, and two had lung tumors. Only 7 out of 14 animals receiving calomel survived beyond one year of age. Three of these had lung tumors, one had carcinoma of the bladder and marked reticular cell hyperplasia of liver, spleen, and lymph nodes, and one had a hemangioma of the liver.

C57BL male mice fed cathartics by stomach tube for 15 months. 26 control mice showed 2 hepatomas, 2 lymphoid neoplasms and no gastrointestinal neoplasms. Nine mice fed calomel plus magnesium sulfate developed 1 hepatoma, 1 lymphoid neoplasm, and 3 gastrointestinal neoplasms. Four additional gastrointestinal neoplasms were found in mice receiving the other cathartics --making a total of 7 gastrointestinal neoplasms in 74 animals (4 were small papillomas of the forestomach, 1 was a squamous cell carcinoma, and 2 were adenomas of jejunum and cecum respectively.

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Part A (continued)

A/HE mice: Most of these died of amyloidosis at a relatively early age (9-15 months) and not much useful information could be obtained.

Significance: The results obtained especially with BALB mice suggest that podophyllum and perhaps also calomel may have weak carcinogenic properties. The liver and the reticuloendothelial system seem to be the tissues most affected.

Proposed Course: No further work is planned in the immediate future but the work with calomel and podophyllum should be repeated using larger groups of animals, and perhaps different strains and species. Use of C3H mice might bring out any latent tendency toward production of hepatomas, and use of less highly inbred (general purpose) strains might accentuate any carcinogenic effect on other organs.

Part B included: No

Serial No. NCI-527
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

NIH-NIH:
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Study of the Physiopathologic Changes in the Tumor-bearing Host and Tumor Lesion

Principal Investigator: Richard A. Malmgren

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 2 1/3
Professional: 1
Other: 1 1/3

Project Description:

Objectives: 1. To study further the properties of the liver mitotic stimulant in tumors. 2. To apply the fluorescent antibody technique to the study of the tumor virus, to autoimmune phenomenon, and the detection of DNA. 3. To evaluate host and cell factors in the metastasis of various mouse tumors. 4. To study the possible interrelationship of carcinogen and virus induced neoplasms.

Methods: 1. The supernatant fraction of various tissues heated at 80°C for 20 minutes were injected subcutaneously into C3H mice. These animals were then challenged with several carcinogens and the tumor incidence and latent period noted. In addition some mice bearing C3HBA mammary tumors were treated with the heated supernatant from C3HBA mammary tumor.

2. Antisera prepared in rabbits against F388 tissue culture cells infected with the polyoma virus was tested by means of the fluorescent antibody technique on F388 tissue culture cells infected with the polyoma virus for antiviral antibody in an attempt to demonstrate the intracellular location of the virus antigen. Sections of salivary gland tumors were also examined after exposure to the same fluorescein labeled antisera.

Chicken and turkey anti-Rouse virus sera was labeled with fluorescein isothiocyanate and tested on sections of Rous sarcoma in an effort to demonstrate the intracellular location of the viral antigen.

Serum from patients with Lupus Erythematosis and other diseases were used in the indirect fluorescent antibody technique on sections, smears and tissue culture cells of various normal and neoplastic tissues of animal and human origin. Blocking tests were also conducted using serum from tumor-bearing animals and humans.

Part A (continued)

3. A number of spontaneous and transplanted mouse tumors have been transplanted into various sites. The metastatic pattern and cell characteristics are then being observed. The tumors being studied include spontaneous mammary tumors, MCA induced sarcomas, transplantable mammary tumors, sarcomas, osteogenic sarcoma and 891 melanoma. (In collaboration with Dr. Elizabeth Chu, Pathologic Anatomy, NCI).

4. Mice injected with the polyoma virus and treated with methylcholanthrene and urethan are being observed for tumor development. (In collaboration with Dr. Clyde Dowe and Dr. Lloyd Law.)

Major Findings: 1) (a) Injection of the liver mitotic stimulating factor in the nucleoprotein rich heated supernatant of C3HBA mammary tumor into mice bearing the mammary tumor has resulted in increased growth of the transplant over that observed in control C3HBA mammary tumor-bearing mice. (b) Injection of the liver mitotic stimulating factor in the heated supernatant from normal mouse liver had no effect on the incidence or latent period of methylcholanthrene induced tumors. (c) Injection of the liver mitotic stimulating factor in the heated supernatant from a transplanted mammary tumor had no effect on the incidence or latent period of methylcholanthrene induced tumors. (d) Injection of the liver mitotic stimulating factor in the heated supernatant from a transplanted methylcholanthrne induced sarcoma markedly decreased the incidence and increased the latent period of MCA induced sarcomas. (e) Injection of the liver mitotic stimulating factor in the heated supernatant from normal liver resulted in a decrease in the number of urethan treated mice having lung tumors and a decrease in the number of tumors per mouse. (f) A similar decrease in the number of mice with urethan induced tumors and a decrease in the number of tumors was found in mice treated with the liver mitotic stimulating factor in the heated supernatant of a transplanted mammary tumor.

2) (a) In a collaborative study with Dr. Alan Rabson and Dr. Giancarlo Rabotti of the Laboratory of Pathology, NCI, the intranuclear location of the polyoma virus in F388 tissue culture cells was demonstrated by the fluorescent antibody technique. The viral antigen could not be [illegible] in sections of polyoma induced salivary gland tumors, however. (b) In collaboration with Dr. Mary Fink of the Laboratory of Biology, NCI, the intranuclear and intracytoplasmic location of Rous sarcoma virus antigen was demonstrated in sections of Rous sarcoma tumors using the fluorescent antibody technique. (c) In collaboration with Dr. Howard Goodman of the National Heart Institute, a technique was worked out for the demonstration of antinuclear antibodies in the serum of patients with Systemic lupus [illegible] and other related diseases. The antinuclear material will react with the nuclei of all cells tested including normal and neoplastic and from human, rabbit, guinea pigs, mice and rats. No antinuclear material was demonstrable in the serum of cancer patients. A blocking effect on the antinuclear [illegible] in Lupus patients sera by sera from cancer patients was not demonstrable.

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Individual Project Report
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Part A (continued)

(a-1) In collaboration with Dr. Howard Goodman of the National Heart Institute, Dr. John Fahey of the General Medicine Branch, NCI, and Dr. George Brecher of the Clinical Pathology Department of the Clinical Center, when serum of lupus patients was fractionated and the fractions tested by the fluorescent antibody technique the activity was found to reside in both the 7S and 18S globulin fractions.

Significance: 1) The studies of the liver mitotic stimulating factor in tumors indicate that this factor tends to promote the growth of an established tumor while it has an inhibitory effect on the carcinogenic effect of methylcholanthrene and urethan. The relationship of these observations to tumor growth and carcinogenesis awaits further study. 2) The intracellular location of the tumor viral antigens is of basic importance to the study and understanding of virus neoplasms. 3) The study of serum factors with antinuclear activity may well contribute to an understanding of the etiology and treatment of the collagen diseases. It is also of interest in terms of the composition of the cancer cell nucleus that it is not distinguishable from the normal by these serum factors. Of equal interest is the absence of a demonstrable difference in the serum nucleoproteins of cancer and normal individuals by this system.

Proposed Course: 1) A study of the growth factors associated with the tumor lesion will be continued with emphasis on improved techniques for detection and characterization. 2) The use of the fluorescent antibody technique for the study of virus tumors will be extended. 3) Further studies of the host cell relationship in the metastatic process will be undertaken.

Part B included: Yes

Part E. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Goodman, H. G., Pobey, J. L., Malmgren, R. A., and Brecher, G.
Separation of Factors in Lupus Erythematosis Serum Reacting with Components of Cell Nuclei.
Lancet 2: 7099: 382-383, 1959.

2. Malmgren, R. A., Fink, M. A., and Mills, W.
Demonstration of the Intracellular Localization of Rous Sarcoma Virus Antigen by Fluorescein Labeled Antisera.
J. Nat. Cancer Inst. In press.

3. Malmgren, R. A., Rabott, G., and Rabson, A. S.
Intracellular Localization of Polyoma Virus Antigen Demonstrated with Fluorescein Labeled Antisera.
J. Nat. Cancer Inst. In press.

Serial No. TGT 530
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-HHH
Individual Project Report
Calender Year 1959

Part A.

Project Title: Developmental Pathology and Function of the Thymus Gland and Thymic Tumors

Principal Investigator: Roger W. O'Gara

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: To obtain information on (a) the function of the thymus gland, (b) the factors involved in the growth of the thymus gland and in the development of epithelial thymomas, (c) the influence of the thymus gland on the induction of lymphoid tumors.

Methods: Surgical procedures and the usual biologic, histopathologic, and radiation techniques.

Major Findings: 1) Serial studies of neo-natal thymic glands transplanted into the spleen of adult mice show that the thymic tissue regenerates from its own cells and not from infiltrated host lymphocytes. 2) Adrenalectomy plus orchiectomy enhanced moderately the growth of intrasplenic thymus transplants but ovarectomy did not. Thymectomy did not appreciably enhance the growth of intrasplenic thymic transplants. 3) One intrasplenic thymic transplant has resulted in a transplantable squamous cell carcinoma. (Now in its 3rd passage.) 4) Additional studies confirm previous reports that leukemia does not develop in irradiated thymectomized C57Bl mice when given intrasplenic transplants of neo-natal thymus glands.

Significance: This is a new experimental method of studying the growth capabilities and reactions of the thymus gland under altered environmental conditions. It is expected to supply information on factors which regulate and control the growth of the thymus gland, and on the development of thymomas and lymphoid tumors.

Part A (continued)

Proposed Course: 1) Additional studies are being made of the comparative effects of neo-natal thymic tissue when transplanted into the liver, spleen, and subcutaneous tissue of thymectomized irradiated mice. 2) The nature of the epithelial cysts found in the spleen after transplantation of neo-natal thymic tissue is being further investigated by comparison with transplants of bronchi, skin, and stomach from neo-natal mice. 3) Groups of irradiated mice will be fed antimetabolites and alkelating agents: (a) during the period of irradiation and (b) during the period following radiation, in an attempt to influence the incidence of radiation-induced leukemia during the preleukemic period. 4) Cholesterol pellets containing hormones will be implanted subcutaneously into mice to study the long-term effect on the thymus.

Part B included: No

Serial No. NCI 534
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Incidence of Induced and Spontaneous Tumors in NIH Swiss Mice Used in Screening Experiments for Potential Viral Induced Human Neoplasms

Principal Investigator: Pearl P. Stanton

Other Investigator: Robert Manaker

Cooperating Units: Laboratory of Biology, NCI

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: The determination of the incidence and character of tumors which occur in the variety of mice used in screening experiments and the detection of unique tumors which may arise in the test mice.

Methods: Dr. Manaker cultures and inoculates newborn mice with control and human tumor inoculated tissue cultures. Dr. Stanton studies the animals for tumors.

Major Findings: Results are in very preliminary stages of analysis. Of 106 tumor-bearing animals (all over 6 months of age) the following percentages have been noted: breast tumors in 20%, pulmonary alveologenic tumors 31%, reticulo-endothelial tumors 21%, uterine leiomyomas 6%, subcutaneous fibrosarcomas 5%. In addition the following rare tumors have been found: 4 adenocarcinomas of the kidney (2 from a group inoculated with the same material and 3 from the same source of material), 2 thyroid adenomas, 1 salivary gland adenoma, 2 mast cell tumors, 1 osteogenic sarcoma, 1 ovarian carcinoma, 1 endometrial carcinoma, 1 pituitary adenoma, 1 cutaneous hidradenoma, and 1 liver hemangioma. The incidence of multiple tumors is remarkably high in this group of animals.

Significance: Such studies are necessary to separate induced tumors from those occurring spontaneously.

Proposed Course: Because the mice are not inbred it will be necessary to study large numbers of mice at various ages to have a clear impression of the normal tumor incidence and variety of lesions which test animals may show. This is a long-term project.

Part B included: Yes

Serial No. NCI [illegible]
1. Laboratory of Pathology
2. Cancer Induc. & Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental Studies on the Effect of Nutritional Deficiencies on the Pathogenesis of Hepatomas in Rats

Principal Investigator: Herschel Sidransky

Other Investigators: 1) Tsunco Enba, Laboratory of Pathology, NCI (Section A (1))
2) Emmanuel Farber, Dept. of Pathology and Biochemistry, Tulane University, New Orleans (Section B (1))
3) V. B. Mitbander, Laboratory of Biochemistry, NCI (Section B (2))
4) Harold P. Morris, Laboratory of Biochem[illegible], NCI (Section C (4))

Cooperating Units:

Man Years (calendar year 1959):
Total: 2 1/3
Professional: 1
Other: 1 1/3

Project Description:

Objectives: To study the pathologic and chemical changes that occur in certain nutritional deficiencies which may be related to the subsequent development of neoplasia in the liver of the rat.

Methods: (A) Kwashiorkor, a widespread protein deficiency disease in infancy and childhood, has been considered possibly to be related to the high incidence of cirrhosis and hepatoma of adults in Africa. Experimentally, we have previously found that young rats force-fed purified diets devoid in certain single essential amino acids, threonine, methionine, or histidine develop many lesions which are similar to those found in kwashiorkor. In order to learn additional information under our experimental conditions, deficiencies in valine or lysine have recently been studied. Also studies are in progress to determine the influence of level of carbohydrate intake on the changes produced by the amino acid deficiencies. In order to simulate more closely the natural diets in areas where kwashiorkor exists, experiments have been carried out in which young rats have been force-fed diets containing certain vegetable proteins as the sole source of protein.

[illegible]

A. Choline deficiency in acute experiments produces a fatty liver in the rat while in chronic experiments hepatoma develops in the rat. The possible relationship between the development of the acute fatty liver and the subsequent development of hepatoma is not clear. In acute experiments the susceptibility to develop fatty livers has been found to vary in certain species. A study has been conducted to learn whether the level of the liver enzyme, choline oxidase, in 8 species of animals and in man, may be related to this altered susceptibility.

In addition, a study has been undertaken in order to obtain a more clear understanding of the relationship of methionine to choline in acute choline deficiency.

Previously it has been shown that rats develop hepatomas when placed on a choline deficient, low methionine diet. More recent studies reveal that ethionine, an ethyl analog of methionine, produces hepatomas in the rat. Ethionine is believed to act as a competitive inhibitor of methionine and thereby produces a relative methionine deficiency state. Methionine but not choline completely inhibits this action of ethionine. To learn more about the mechanism by which interference with methionine metabolism can induce hepatomas in rats, current experiments have been started in which animals have been placed on a methionine deficient, but adequate choline, diet.

B. Ethionine has recently been reported to produce hepatomas in the rat. Although much experimental work has been performed on the acute actions of ethionine, little is known about its action in chronic experiments leading to hepatoma. Several studies have been started to study certain aspects in the development of hepatomas. The influence of partial hepatectomy before placing rats on ethionine is under investigation. P-Hydroxypropiophenone (PHP) has been described to inhibit the development of liver tumors caused by butter yellow. The affect of PHP on hepatoma formation due to ethionine is presently being studied.

Ethionine and N-2-fluorenylacetamide (N-2-FAA) have each been shown to induce hepatomas in rats. Male rats are more susceptible than females to the carcinogenic action of N-2-FAA while no sex difference has been reported with ethionine. The early histopathological changes produced by each agent have been reported to be similar. For this reason, experiments have been started to determine whether these agents fed together in the diet may act synergistically in the development of hepatomas and to observe whether a sex difference will be found.

Studies have been started to learn more about the sex difference in liver tumorogenesis due to N-2-FAA. Rats of both sexes of the Osborne-Mendel strain and Buffalo strain have been sacrificed at intervals in order to study the early histologic changes in the liver.

Part I (continued)

Major Findings: A. 1) One publication describes the chemical pathology of acute valine and lysine deficiencies. 2) Preliminary studies appear to indicate that the level of carbohydrate intake is important in producing the pathologic changes described in the above and previous publications. If the carbohydrate intake is lowered while all other nutrients are kept constant, few pathologic changes result. 3) Rats force-fed certain vegetable proteins (corn, cassava, milo, wheat, and rice) for three days develop many pathologic changes similar to those described with previous amino acid deficiencies.

B. 1) One publication describes the various levels of liver choline oxidase in man and in 8 species of animals. 2) Preliminary studies reveal that growth and pathologic changes due to choline deficiency are highly dependent on the level of dietary methionine intake. 3) Chronic experiments in which rats have been placed on a methionine deficient diet have now been in progress for 11 months. Rats will be followed for an additional 12 months.

C. 1) Experiments in which rats were partially hepatectomized and then placed on an ethionine diet, have now been followed for six months with no findings to date. 2) Experiments in which rats were fed ethionine with and without PHP have been followed for nine months. Preliminary findings indicate that PHP does not alter the early morphologic changes due to ethionine. 3) Experiments in which rats were fed control, ethionine, N-2-FAA, and ethionine plus N-2-FAA diets were observed. Male and female rats fed the ethionine plus N-2-FAA diet died with hepatomas at an earlier time than in all other groups. 4) Experiments in which rats were fed N-2-FAA and sacrificed at monthly intervals reveal that the histopathologic changes are different in the male than in the female. The male rat develops changes earlier and of a different type than in the female.

Significance: Up to the present time the only nutritional deficiency which has been found to produce liver cancer in experimental animals is that of choline deficiency. It would be of much interest and importance to determine whether other deficiencies such as chronic amino acid or protein deficiencies might under certain conditions produce hepatomas in experimental animals.

Proposed Course: Rats placed on different diets will be sacrificed at varying intervals. Detailed pathologic studies will be conducted and the pathologic findings will direct the course of the chemical investigations.

(Note: In addition to work described above, collaborative work related to liver carcinogenesis is being carried out with Dr. W. P. Morris. Details will be given in his report.)

Part B included: Yes

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project

1. Sidransky, Herschel, and Baba, Tsuneo
Chemical Pathology of Acute Amino Acid Deficiencies: III. Morphologic and Biochemical Changes in Young Rats Fed Valine- and Lysine-Devoid Diets.
J. Nutrition. In press.

2. Sidransky, Herschel, and Farber, Emmanuel
Liver Choline Oxidase Activity in Man and in Several Species of Animals.
Arch. Biochem. Biophys. In press.

3. Sternberg, William H., Sidransky, Herschel, and Ochsner, Seymour
Primary Malignant Lymphomas of the Lung.
Cancer 12: 806-819, 1959.

4. Sidransky, Herschel, and Friedman, Lorraine
The Effect of Cortisone and Antibiotic Agents on Experimental Pulmonary Aspergillosis.
Am. J. Path. 35: 169-183, 1959.

5. Pearl, Maurice A., and Sidransky, Herschel
Fungus Infections of the Lung in Patients Treated with Corticosteroids and Antibiotics
Bull. Tulane M. Fac. 19: 35-42, 1959.

6. Sternberg, William H., and Sidransky, Herschel
Pathologic Observations on Primary Malignant Lymphomas of the Lung.
Bull. Tulane M. Fac. 19: 1-10, 1959.

Honors, Awards relating to this project:

Member, American Society for Experimental Pathology, April 1949
Member, American Association of Path. & Bact, April 1959

1. Laboratory of Pathology
2. Cancer Pathology Section
3. Bethesda, Maryland, and
New Orleans, Louisiana

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Geographic pathology field studies on urinary bladder cancer in New Orleans, Louisiana

Principal Investigator: Lucia J. Dunham, Harold F. Dorn, and Harold L. Stewart

Other Investigators: Alan S. Robson, Louis B. Thomas

Cooperating Units:

Man Years (calender year 1959):
Total: 1 5/6
Professional: 1 1/3
Other: 1/2

Patient Days (calender year 1959):

Project Description: Field studies on Urinary Bladder Cancer, New Orleans, Louisiana.

Objectives: The objective of this project is to complete an exhaustive questionnaire study of patients with urinary bladder cancer in a southern U.S.A. city with a high incidence of bladder cancer compared to two other southern cities. (Dorn and Cutler, Morbidity from Cancer in the United States, Public Health Monograph No. 56). The data will be analyzed with a view to identifying factors associated with cases of urinary bladder cancer. Basic data on the incidence and pathologic (microscopic) diagnosis of the cancers are being obtained.

Methods: For the area under study and the period of study all patients possible with the diagnosis of urinary bladder cancer are interviewed, and an equal number of control patients as well. The data recorded include residence, occupation, medical and surgical history, and information about diet and about the use of alcoholic and non-alcoholic beverages and of tobacco. Records are kept of the small number of patients not interviewed, their diagnoses, and the reasons for failure to interview. A coding statistician and one medical social worker interviewer are currently employed in the project. Interviewing was started in New Orleans on 15 February 1958.

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Individual Project Report
Calendar Year 1959

Part A (continued)

Major Findings: Data from about 300 cancer patients and an equal number of control patients have been reviewed and coded. It is estimated that pathologic material will have been examined in about 90 percent of the cases at the date on which this report is submitted.

Significance: The significance of the study lies in the hope of supplying a sound scientific basis for the understanding of factors in habit or environment as they may be related to a high incidence of urinary bladder cancer in a specific geographic area.

Proposed Course: It is planned to continue the study for a total of three or more years. Data will be tabulated and analyzed and a report will be prepared for publication.

Part B included: No

1. Laboratory of Pathology
2. Cancer Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Geographic pathology field studies on uterine cancer in New York City, Israel, and Washington, D.C.

Principal Investigator: Lucia J. Dunham, Harold F. Dorn, and Harold D. [illegible]

Other Investigators: John H. Edgcomb, Louis B. Thomas

Cooperating Units:

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 5/6
Professional: 1/3
Other: 1/2

Project Description: Field studies of uterine cancer (cervix and corpus) in New York City, Israel, and Washington, D.C.

Objectives: The objective of this project is to complete an extensive questionnaire study of women with uterine cancers in different geographic areas and racial groups, and to analyse the data obtained with a view to identifying factors suspected of predisposing to or causing female genital cancers. Also, basic data on the incidence and pathologic (histologic) diagnoses of the cancers are obtained.

Methods: For the area under study and the period of study all patients possible with the diagnosis of uterine cancer are interviewed, and an equal number of control patients as well. The confidential data recorded include social, medical, and surgical history, and menstrual, marital, and pregnancy data. Records are kept of the small number of patients not interviewed, their diagnoses, and the reasons for failure to interview. Pathologic material is studied in all cases.

A coding statistician and one medical social worker interviewer are currently employed in the project.

Major Findings: 1. Data from 3,514 cancer and control patients, Jewish and non-Jewish white, in New York City and Israel have been reviewed, coded, tabulated and analysed. Pathologic material has been examined in the cancer cases. A report has been prepared entitled Cancer of the Uterus, Part I, Jewish and non-Jewish white women in New York City and Jewish women in Israel. The seven authors, including Dr. J. Casper, Petah Tiqva, Israel and Dr. A. Symeonidis, Thessaloniki, Greece, are currently editing the manuscript preliminary to publication. Twelve additional critics are also reviewing the manuscript which will be published in the near future as a U. S. Public Health Service Monograph.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A (continued)

Major Findings: 2. Part II. Negro women in New York City. Data from about 500 cases of cancer of the uterus in non-white women in New York City have been reviewed and coded, together with an equal number of control cases. Pathologic material has been examined in all cancer cases. Data are ready to be transferred to punch cards for machine analysis.

3. Part II. Negro women in Washington, D.C. Data from about 400 cases of cancer of the uterus in Negro women and from about 400 control cases will be assembled by February 1 or March 1, 1960. Data from the entire Part II study of Negro women will be coded and analyzed during 1960.

Significance: The significance of the study lies in the hope of supplying a sound scientific basis for the understanding of factors of custom, habit, or environment as they may be related to relatively high frequencies of uterine cancers in some groups of women as contrasted to intermediate or relatively low frequencies in other groups. In addition, a critical evaluation of the study, prepared at the same time as the report, should provide a useful commentary on techniques in the epidemiology of cancer.

Proposed Course: It is planned to publish the report of Part I of the study, to prepare the report of Part II for publication, and to explore other techniques than were used in these retrospective studies. The other techniques to be considered include: (1) a prospective study of cancer of the uterus in a large group of healthy persons, such as enrollees of the Health Insurance Plan of Greater New York, and (2) a comparative genetic study of rates of cancers of the uterus in white women, inbred and outbred triracial isolate groups of women and Negro women.

Part B included: No

1. Laboratory of Pathology
2. Cancer Pathology Section
3. [illegible], Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Geographic Pathology. Experimental studies relating to the geographic pathology of mouth cancer.

Principal Investigator: Lucia J. Dunham

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959): Total: 2/3 Professional: 1/3 Other: 1/3

Patient Days (calendar year 1959):

Project Description: Experimental studies on mouth cancer.

Objectives: The objective of this project is to examine the mucous membrane of the hamster cheek pouch for proliferative changes that may develop when test substances are chronically maintained in contact with these surfaces. Substances used are environmental materials suspected of being mildly carcinogenic for man.

Methods: The pellets which have been prepared for insertion in the right cheek pouch of the hamster include the tobacco, lime, gambir, areca nut, and pepper leaf of the Far Eastern "betel quid", the plastic material used in preparing "false" teeth, and tobacco tars and control materials. A technical assistant is retained part-time for help in the study.

Major Findings: The operation for inserting pellets in the pouch has been performed on more than 400 animals. No animals survive at the present time. Gross and microscopic lesions of the pouch wall have been observed in about 15 of the several hundred experimental animals examined. There have been no malignant neoplasms among these. It has been demonstrated that neoplasms can be initiated in the pouch wall by using pellets containing a known carcinogen. When tissues from all the animals have been examined all findings in the pouches and other pathologic changes will be reviewed and a report will be prepared. (See current studies, Serial No. NCI 53). Dr. Katherine Herrold).

[illegible]
Individual Project Report
Calendar Year 1959

Part A (continued)

Significance: The significance is to reach an improved understanding of chronic irritant factors as they may relate to cancer of the mouth in man. In addition it will be ascertained whether it is desirable to do further studies using modifications of the technique described.

Proposed Course: The cheek pouch with the test pellet, and the opposite pouch, are both examined when the hamster is sacrificed or dies. The findings will dictate the future conduct of the investigation. A report will be prepared for publication. One or more experimental approaches of possible use for the bio-assay of weak carcinogens will be explored.

Part B included: No

Serial No. NCI 524
1. Laboratory of Pathology
2. Cancer Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Polyoma Virus in Serum-Free Tissue Culture Systems

Principal Investigator: Alma S. Rabson

Other Investigators: Frances Y. Legallais

Cooperating Units:

Man Years (calendar year 1959): Patient Days (calendar year 1959)
Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives: Studies of polyoma virus in cultures of F388 D1 cells grown in serum-free medium have been undertaken to attempt to learn about the action of this virus on proliferating cells in a growth medium free of serum antibodies and non-specific serum viral inhibitors.

Methods: Polyoma virus has been grown in cultures of milk-adapted F388 D1 cells using conventional tissue culture virological methods and a cytopathic effect has been demonstrated. The virus-host cell system has been studied cytochemically in collaboration with Dr. Robert Love; with fluorescent antibody techniques in collaboration with Drs. Malmgren and Rabotti; with measurement of DNA by Feulgen microspectrophotometry in collaboration with Drs. Rabotti and Malmgren, and with the electron microscope in collaboration with Dr. William Ranfield.

Major Findings: In the cytochemical studies carried out with Dr. Love, an abnormal ribonucleoprotein has been demonstrated in the nucleus of milk-adapted F388 D1 cells infected with polyoma virus. By means of the fluorescent antibody methods, an antigen associated with polyoma virus has been demonstrated in the nucleus of infected cells. Microspectrophotometrically, Rabotti has found no increase in nuclear DNA in infected cells although there is an increase in nuclear volume. Ranfield has found large numbers of particles consistent in size with polyoma virus within the nuclei of infected F388 D1 cells. All of these findings are consistent with but by no means conclusive proof of intranuclear replication of an RNA type virus in milk-adapted F388 D1 cells infected with polyoma virus.

Part A (continued)

Significance: An understanding of the mechanism of action of polyoma virus in vitro will probably be of great importance to our understanding of viral neoplasia. The systems for the isolation of polyoma virus may be good experimental models for the isolation of comparable agents from human tumors.

Proposed Course: The studies of the cytopathology of polyoma virus in BSC 01 cells in serum-free medium will be continued. Systems for the growth of the cells and virus in a completely chemically defined medium are being developed and should facilitate biochemical studies of the virus-host cell system. Attempts to find a proliferative virus-host cell system in culture have been unsuccessful but will be continued.

Part B included: Yes

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Rabson, A. S., and Legallais, F. Y.
Cytopathogenic Effect Produced by Polyoma Virus in Cultures of Mouse-adapted Murine Lymphoma Cells (Strain P388 D1)
Proc. Soc. Exp. Biol. & Med. 100: 229-233, 1959.

2. Love, R., and Rabson, A. S.
Cytochemical Studies of Mouse-adapted Murine Lymphoma Cells (Strain P388 D1) Infected with Polyoma Virus
J. Nat. Cancer Inst., in press.

3. Malmgren, R. A., Rabotti, G., and Rabson, A. S.
Intracellular Localization of Polyoma Virus Antigen Demonstrated with Fluorescein Labeled Antisera.
J. Nat. Cancer Inst., in press.

3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Attempts to Isolate Oncogenic Viruses from Human Tumors

Principal Investigator: Alan S. Rabson

Other Investigators: Frances V. Legallais

Cooperating Units:

Man Years (calendar year 1959)
Total: 1 1/3
Professional: 2/3
Other: 2/3

Project Description:

Objectives: Attempts are being made to isolate oncogenic viruses from human tumors.

Methods: Extracts of fresh human tumors are prepared and inoculated into tissue cultures of human cells, into newborn Syrian hamsters and into fetal hamsters in utero. Blind passages of the tissue culture fluids are made at intervals to fetal and newborn hamsters. The tissue cultures used have been grown in serum-free medium to avoid possible presence of serum antibodies or serum viral inhibitors.

Major Findings: A strain of human epidermoid carcinoma cells has been isolated and grown for two years in a serum-free medium containing autoclaved non-fat milk. Cultures of this cell strain are inoculated with extracts of fresh human tumors and observed for possible cytopathic effects. The study has been in progress for less than one year and no cytopathic agents have been isolated yet. The hamster inoculation studies have been in progress for 8 months and only one inoculated animal has developed a tumor.

Extracts of 33 human tumors have been inoculated thus far. These include Wilms tumor of the kidney, mesothelioma of the pericardium, Hodgkin's disease, squamous cell carcinoma of the oral cavity, embryonal rhabdomyosarcoma, ganglioneuroblastoma, synovial sarcoma, undifferentiated carcinoma of the paranasal sinuses, fibrosarcoma, hepatoblastoma, malignant melanoma, choriocarcinoma, and various types of leukemia.

Unit 3 (continued)

Significance: The first demonstration of a human oncogenic virus will be of great importance in the study of human cancer.

Proposed Course: The immediate studies with cultures of selected human tumors using the tissue culture system and animal systems described above will be terminated.

Part 2 Included: Yes

Part B: Honors, Awards, and Publications

1. Growth of Human Epidermoid Carcinoma in Tissue Culture using Non-fat Milk Medium.
A. S. Rabson and P. V. Angelakis
Archives of Pathology 53: 343, 1952

Serial No. NCI 532
1. Laboratory of Pathology
2. Cancer Pathology Section
3. Bethesda, Maryland

NIH-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Brain Tumors Produced in Hamsters by Intracerebrally Inoculated Polyoma Virus

Principal Investigator: Alan S. Rabson

Other Investigators: Ruth L. Hirschstein, Division of Biological Sciences, NIH

Cooperating Units:

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives: The response of Syrian hamsters to intracerebral inoculation of polyoma virus has been studied in an attempt to learn about the types of brain tumors that may be produced by a mammalian oncogenic virus and to learn some of their biological properties.

Methods: Pools of polyoma virus grown in milk-adapted F3DH mouse cells have been prepared and titrated by serial ten-fold dilutions in the same cell system. Varying amounts of virus have been inoculated intracerebrally into young Syrian hamsters. The animals have been carefully observed for evidence of intracranial disease and complete autopsies have been performed on animals found dead or moribund. Portions of brain tumors have been preserved aseptically for virus isolation studies.

Major Findings: Some of the findings have been presented in a paper that is now in press. The summary of this paper follows:

"Intracranial sarcomas have been produced in Syrian hamsters inoculated intracerebrally with polyoma virus. These mesenchymal tumors appeared to arise from connective tissue in the pia mater on the surface of the brain and in the sulci, and from the adventitia of small meningeal and cerebral vessels. The tumors have developed in animals inoculated on the day of birth and at 8, 9, and 12 days of age, and they have been found in animals which have died as soon as 31 days after inoculation. Histologically,

Part A (continued)

The intracranial sarcoma of the hamster produced by polyoma virus resembles the sarcomatous lesions of the brain produced in the young chicken by intracerebrally inoculated Rous sarcoma virus. The hamster tumors also resemble the rare mesenchymal tumors of the brain in man which have been called leptomeningeal sarcomas. These observations suggest that attempts to isolate oncogenic viruses from human leptomeningeal sarcomas might be successful."

Significance: The demonstration of meningeal sarcomas produced by polyoma virus in hamsters suggests the possibility that similar tumors in man may also have a viral etiology. Attempts are being made to obtain tumor from human leptomeningeal sarcomas for virus isolation studies.

The virus-host system of intracerebrally inoculated polyoma virus in young Syrian hamsters may be a useful one for studies of the mechanisms of viral tumor formation. It is suitable for histogenetic studies and for dose response studies. It may be a more precise in vivo assay method than the subcutaneously inoculated newborn mouse or hamster since death should be more rapid and certain with a space-occupying intracranial tumor. Studies to evaluate the usefulness of the system are now in progress.

Proposed Course: Precise studies of the histogenesis of the intracranial lesions are now in progress. In vivo infectivity titrations of virus pools with known tissue culture infectivity and known hemagglutinin content are also in progress. The histological types of tumors produced with small inocula over long periods of time will be of considerable interest since the possibility of glioma production by the virus may not have been eliminated. Tissues are being preserved aseptically from brain tumors produced by varying doses of virus and attempts will be made to titrate the amount of virus in these tumors to determine whether there is any relationship between size of inoculum and amount of virus in the tumor.

Part B included: Yes

Part B. Honors, Awards, and Publications:

1. Intracranial Sarcomas Produced by Polyoma Virus in Syrian Hamsters.
A. S. Robson and B. L. Kirschstein
Archives of Pathology. In press

1. Laboratory of Pathology
2. Cancer Pathology Section
3. Bethesda, Maryland

Individual Project Report
Calendar Year 1959

Project: Effect of species variation on the oncolytic properties of Coxsackie B3 virus on heterotransplants of HeLa cells

Principal Investigator: R. Gerald Suskind

Other Investigator: Robert Love

Man Years (calendar year 1959):
Total: 1/6
Professional: 1/6
Other: -

Project Description: An attempt was made to compare 2 clonal strains of Coxsackie B3 virus, one oncolytic and the other non-oncolytic, in heterotransplants of HeLa in mice with their behavior in heterotransplants of HeLa cells in the chick embryo. The project has been temporarily abandoned because of the difficulty of producing reproducible HeLa tumors in the chick embryo.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part I.

Project Title: The cytopathological and cytochemical changes of parainfluenza virus infection in different cell systems.

Principal Investigator: L. Gerald Kahlon

Other Investigators: Robert Ross

Man Years (calendar year 1959):
Professional: 1/6
Technical: 1/6

Project Description:

Objectives: The cytopathological changes produced by infection with a recently described myxovirus, particularly with regard to changes in nucleic acids and nucleoproteins are under study in different cell systems. Rates of virus synthesis as measured by the hemadsorption technique are correlated with changes in nucleic acid and nucleoprotein distribution, particularly by toluidine blue-molybdate staining. Differences have been observed in infected HeLa cells, where nuclear degeneration and syncytial type giant cell formation is prominent, and in infected monkey kidney cells, where the virus proliferates without causing cellular degeneration. The protein and nucleic acid alterations are compared in these cell systems. Autoradiographic and fluorescent antibody studies to correlate these changes with sites of virus synthesis are planned. An opportunity to study the cytological aspects of a virus host cell relationship in a system in which the same DNA containing virus can live in symbiosis with one cell type or another, a relationship which is of basic interest in the pathogenesis of cancer.

Part II. A manuscript is in preparation for the April [illegible]

Serial No. ZCH 539
Laboratory of

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Further observations on nucleoprotein staining with toluidi
blue molybdate

Principal Investigator: Robert Love, M.D.

Other Investigator: R. Gerald Suskind, M.D.

Cooperating Units:

Man Years (calendar year 1959): Patient Days (calendar y
Total:
Professional:
Other:

Project Description: (See report of Dr. Love, NCI-523). In addition to the
3 stages already described a 4th stage was noted
in Hela cells and ascites tumor cells. Methods concerning nucleoprotein
were observed in the Golgi area of Hela cells.

Part B. Manuscript in preparation.

Individual Project Report

Item 1

Project Title: Comparison of the oncolytic properties of Coxsackie [illegible] for mouse lymphoma cells (P388) in vitro and in vivo

Principal Investigator: [illegible]

Other Investigator: Robert Love

Man Years (calendar year 1956):
Total: 1/2
Professional: 1/6
Other: 1/3

Project Description: Previous studies of Coxsackie B_3 virus in heterotransplants of HeLa cells have shown their serial passage in mice to result in adaptation for oncolytic properties of this virus, a process which is attended by passage in vitro. An attempt was made to reproduce this phenomenon with Newcastle Disease Virus and mouse lymphoma cells (P388) in mice and in vitro. It was found that an unadapted [egg adapted] strain of Newcastle Disease Virus was oncolytic for P388 tumors without further adaptation. [illegible] a specific oncolytic [illegible] failed to develop. [illegible] further details not ready at this time].

Item 2 Presentation of a case of essential pulmonary hemosiderosis Clinicopathologic Conference, Clinical Center.

Case report: (In preparation) Carcinoma of the liver associated with hemihypertrophy in a 22 month old infant.

Case report: (In preparation) Congenital Toxoplasmosis.

Serial No. [illegible]
1. Laboratory of Pathology
2. Pathological Technology
3. Bethesda, Maryland

Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pathological Technology Section

Principal Investigator: Joseph M. Albrecht

Other Investigators:

Cooperating Units:

Man Years (calendar year 1959):
Total: 30
Professional: 4
Other: 26

Project Description:

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for all the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

November 1, 1958 - October 31, 1959

Number of investigators	73
Number of pieces of tissue	215,993
Number of bottles of tissue	28,757
Number of blocks cut	112,404
Number of blocks cut serially	1,602
Number of frozen blocks cut	1,259
Number of autopsies recorded	21,430
Number of recuts	1,167
Number of slides stained H & E	133,938
Number of slides stained special	20,020
Number of unstained slides	11,155

Part A (continued)

Photographic Service rendered to the Laboratory of Pathology November 1, 1958, to October 31, 1959.

GROSS	
Black & White	223
Color	85
MICROS	
Black & White	1043
Color	700
LANTERN SLIDES	
Black & White	200
Color	450
PRINTS	
4 x 5	3943
8 x 10	101
Mounted	366

Part B included: No

Serial No. NCI-904
1. Laboratory of Physiology
2. Office of the Chief
3. Department of Biology
Princeton University
Princeton, N. J.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Ultraviolet Light and Carcinogenesis

Principal Investigator: Harold F. Blum

Cooperating Units: Department of Biology, Princeton University
Princeton, New Jersey

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: 0

Project Description:

I. Quantitative Studies of Carcinogenesis

A. Carcinogenesis by ultraviolet light.

Last year a quantitative model was formulated which described experimental data on the induction of cancer in albino mice by ultraviolet light. The process was shown to be continuous, cumulative and essentially irreversible and non-threshold. Equations were derived describing this induction in terms of the concept that the tumor consists of an increasing number of clones whose rate of proliferation is accelerated by repeated doses of ultraviolet light. Experimental data support this conception. By this model the initial volume of these tumors could be calculated. This volume calculated to be smaller than a tumor cell, which indicated that the basic replicated unit governing the growth of these tumors is of subcellular dimensions. (See attached bibliography references 4-7).

B. Carcinogenesis by other agents.

Since cancer may be considered as a growing part of its host, it should be influenced by factors that affect the overall

growth of the host. Hence, this lends a degree of uncertainty to the interpretation of results of cancer studies in which time of appearance, the incidence in a population, therapeutic action on cancer, the action of endocrines, etc. are concerned (3).

A paper entitled "Quantitative Aspects of Cancer Induction and Growth: As Illustrated in Carcinogenesis by Ultraviolet Light" (8) was called to the attention of Congresswoman Leonor K. Sullivan of Missouri as having a bearing on the problem of tolerance limits for suspected carcinogenic dyes in food. Upon the request of Congresswoman Sullivan, I replied explaining the infeasibility of setting tolerance limits for carcinogens. This letter appears in the Congressional Record of August 27, 1959.

Congresswoman Sullivan's letter stimulated me to return to quantitative analysis of data on other carcinogens, which I had attempted earlier without much success. The particular point in question is whether the action of chemical carcinogens, like that of ultraviolet light, may be cumulative. The latter appears true, but I have met again with little success in a quantitative analysis because most of the data have not been taken in a way that will permit accurate analysis of this aspect. The attempt at analysis will continue, but it seems clear that to obtain really adequate data would require extensive, carefully planned experiments, involving the participation of considerable personnel on a long term basis.

The possible relationship of the findings with ultraviolet light to the "fall-out" problem has been discussed in a brief paper to appear soon in Science (12) entitled "Environmental Radiation and Cancer".

II. Studies of the Acute Effects of Ultraviolet Light on Skin

A single dose of ultraviolet light evokes a strongly hyperplastic response from the epidermis. The proliferation of the epidermal cells for a time reaches rates that are higher than those found for the cancers that develop after repeated doses. The possible relationship between these two things has been of interest to me for a long time, and it has seemed possible that some information about the carcinogenic process might be contained therein. Five years ago I began studies on the effect of single doses of ultraviolet light on the ears of strain A mice, which have proceeded slowly because of technical difficulties and limited maintenance. However, it was possible to make a qualitative and semi-

quantitative study which has been briefly described in an article appearing in the Journal of the National Cancer Institute (3).

The quantitative aspect of the study has been recently pushed forward with considerable success by Mr. Gerald Soffen, as the basis of his thesis for the doctorate, under my supervision. Mr. Soffen has held, since September 1958, a Public Health Service pre-doctoral Fellowship.

This study has provided consistent quantitative results, parts of which may be described by relatively simple equations. Mr. Soffen and I, and latterly Dr. Roger Pickham, have attempted to find a meaningful general equation to describe all the results, but with little success up to now. The attempt will continue, however, and it seems likely that success will ultimately be achieved. But in the meantime, partial analysis shows some interesting relationships which seem to have basic significance.

Attention focusses on the proliferation of the cells of the epidermis, which may be measured accurately by counting mitotic figures at different times after the exposure to ultraviolet light. The accompanying figure shows an example of the relationship found. The curves represent the incidence of mitosis, properly corrected and plotted on a logarithmic scale against the time subsequent to exposure. A number of curves are included which represent different dosages of radiation from an intermediate pressure mercury arc. A similar series of experiments with exposures to a low pressure mercury arc yielded the same kind of curves; but with a somewhat different dosage relationship, which seems explainable on the basis of difference in penetration of the radiation from the two sources.

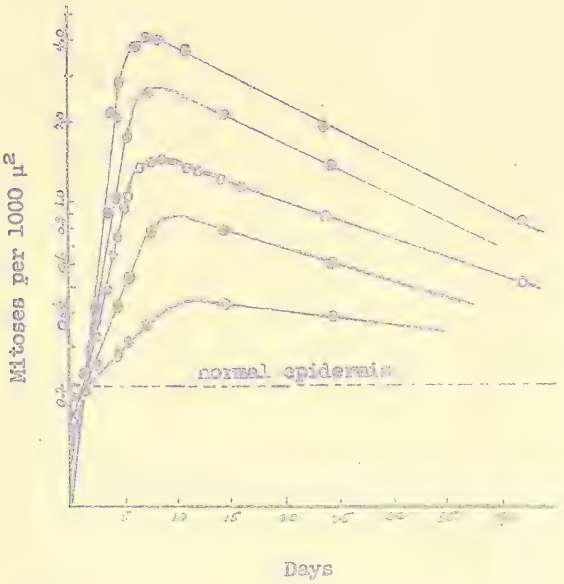


Figure 1. Course of epidermal proliferation following exposure to ultraviolet radiation as measured by the incidence of mitosis. The mitotic counts have been corrected in terms of the number per unit area of skin; this takes account of the increase in total population due to increase in number of cells, since growth only occurs in one dimension.

The effects of five different doses of radiation are represented by the five curves, the effect increasing with dose. Early in their course the curves are straight lines, indicating that the proliferation rate of the cells is constant. Later the rate falls off, passes through a maximum, and then the curves again become straight lines, but with negative slope.

It has generally been assumed, due largely to scattered studies on human skin that exposure to ultraviolet light is followed by a latent period before hyperplasia or other changes begin. It is to be noted that the curves all reach the base line for mitosis in normal skin at about 1.5 days after the exposure, and this might on first examination be thought to represent a latent period, particularly if only one curve were examined. However, the crossing of the base line at the same point, and the finding that the mitotic counts prior to that time (1.5 days) are lower than those for the normal tissue, indicate that the ultraviolet light has rendered a portion of the epidermal cells incapable of dividing. Pending a more complete mathematical analysis, these curves seem best explained as the result of influence of injured or moribund cells on the surviving proliferating cells. Thus the higher the dose of ultraviolet radiation, the greater the initial destruction of cells (resulting in a lower starting point on the ordinate), and the greater the initial rate of cell proliferation.

The swelling of the cells and their nuclei after the dosage with ultraviolet light, indicates increased permeability and suggests the possibility that the increased rate of proliferation depends upon the release of material from the moribund cells which can be used by the survivors in their replication. Such an explanation is in accord with tentative suggestions regarding hyperplasia which appear in my recent book (1), and also with a working hypothesis of carcinogenesis that is presented therein. The latter assumes that it is the accumulation of templates, called for convenience "temps," in some of the tissue cells, which confers on them the inherited rapid rate of proliferation that characterizes the clones of cells making up a cancer. With this possibility in mind, experiments are now being undertaken to study the effect of a second dose of radiation following the first. The analysis of the findings already obtained on single doses will, of course, be continued, since this must form a necessary basis, for the exploration of successive doses.

III. Studies of the Effect of Non-ionizing Radiation on Permeability

Studies of permeability changes in red blood cells following irradiation with ultraviolet light and with visible light after photosensitization, have been going on in this

laboratory for some time. A few years ago, Dr. John Cook, in studying hemolysis by ultraviolet light, discovered here an unexpected relationship, in that the hemolysis time varies as the square of the dose. Later while Dr. Cook held a Public Health post-doctoral Fellowship we collaborated in studies, the results of which have appeared this year in an article in the Journal of Cellular and Comparative Physiology (9). The dose-squared relationship has not been satisfactorily explained as yet, and the question of mechanism has become more important with our finding that the same relationship holds for photodynamic hemolysis, where an entirely different photochemical reaction is at base.

Because of the possible relationship of permeability changes to carcinogenesis, I have attempted to find a theoretical explanation of the dose-squared relationship. The basic idea is that both membrane permeability and colloidal osmotic pressure are increased progortionately by the radiation. The analysis is not yet in shape to present for criticism, but I hope to write it up in the near future.

An account of earlier studies on permeability changes with photodynamic action, in collaboration with Professor J. A. Green of Rutgers University, and Professor Arthur K. Parpart of Princeton, will appear in an early issue of the Journal of Cellular and Comparative Physiology (11). Prehemolytic changes in photosensitized rabbit red cells were studied. Na-K exchange and glucose disappearance were studied while the cells were being exposed to brief periods of visible light in air. Brief periods of photosensitization markedly accelerated Na uptake and K loss while only slightly increasing the rate of glucose disappearance. It appears likely that the acceleration of cation exchange with photosensitization is the result of cell surface alteration rather than of an effect on cell metabolism.

Significance to the program of the Cancer Institute: To obtain a better understanding of the mechanism of carcinogenesis by ultraviolet light and of acute effects of ultraviolet light on the skin.

Proposed course of project: To continue this program.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Blum, H. F.: Carcinogenesis by Ultraviolet Light: An Essay in Quantitative Biology. Princeton University Press. 1959, pp. 340.

2. Blum, H. F.: Time's Arrow and Evolution. (ed. 2) Princeton University Press, 1959. (Paper bound edition.) (First edition 1955).

3. Blum, H. F., Butler, E. G., Dailey, T. H., Daube, J. R., Mawe, R. C., and Soffen, G. A.: Irradiation of mouse skin with single doses of ultraviolet light. J. Nat. Cancer Inst. 22: 979-993, 1959.

4. Blum, H. F.: Coordinated growth of body parts and cancer. J. Nat. Cancer Inst. 23: 229-239, 1959.

5. Blum, H. F.: On the mechanism of cancer induction by ultraviolet radiation. II. A quantitative description and its consequences. J. Nat. Cancer Inst. 23: 319-335, 1959.

6. Blum, H. F.: On the mechanism of cancer induction by ultraviolet radiation. III. The growth curve. J. Nat. Cancer Inst. 23: 337-342, 1959.

7. Blum, H. F.: On the mechanism of cancer induction by ultraviolet radiation. IV. The size of the replicated unit. J. Nat. Cancer Inst. 23: 343-350, 1959.

8. Blum, H. F.: Quantitative aspects of cancer induction and growth: As illustrated in carcinogenesis by ultraviolet light. American Scientist 47: 250-260, 1959.

9. Cook, J. S., and Blum, H. F.: Dose relationships and oxygen dependence in ultraviolet and photodynamic hemolysis. J. Cell. Comp. Physiol. 53: 41-60, 1959.

10. Blum, H. F., Butler, E. G., and Schmidt, S. E.: Regeneration of limb abnormalities after ultraviolet irradiation. J. Cell. Comp. Physiol. 52: 177-186, 1958. (While dated 1958, this paper appeared in 1959).

11. Green, J. A., Blum, H. F., and Parpart, A. K.: Prehemolytic studies of photosensitized erythrocytes. J. Cell. Comp. Physiol., 1959. (This paper has been scheduled for the August issue of this journal, which has not yet appeared.)

12. Blum, H. F.: Environmental radiation and cancer. Science. (This paper should appear before the end of this year.)

Honors and Awards relating to this project:

Seminars and Symposia

April 7 — Seminar "Ultraviolet Light and Cancer," Department of Biology, Rutgers University.

April 15 — Lecture "Quantitative Aspects of Growth and Spread of Cancer Induced by Ultraviolet Light," at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics, Atlantic City.

April 16 — Seminar "Back of Natural Selection," Swarthmore University.

November 17 — Sigmund Pollitzer Lecture "Carcinogenesis by Ultraviolet Light; Experiment and Interpretation," New York University Medical School.

December 2 — Paper "Experimental Photobiology, with Special Reference to Studies on Skin," at Symposium on Sunlight and the Skin under auspices of the Committee on Cosmetics of the American Medical Association, Clinical Meeting, Dallas, Texas. I also appeared in a television broadcast in connection with this meeting.

Serial No. NCI-918
Laboratory of Physiology
Office of the Chief
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Research Development Projects in the Field of Electronics

Principal Investigator: Nathan Coffey

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: To design new electronic equipment as the needs of the Laboratory arise; to modify existing electronic equipment to meet the changes required in an experiment; and to supervise the research development projects in the field of electronics.

Major Findings:

(1) Modification of recording spectrophotometer to permit fluoroscopic determinations to be made. (Dr. James C. Reid)

(2) Consulted with investigator and manufacturer of transducers used in gas analysis cells in metabolism studies in effort to further improve operating characteristics. Additional transducers producing an opposite reaction are being checked in an effort to increase available sensitivity through an additive effect. (Dr. A. W. Pratt)

(3) Adiabatic cage previously constructed further refined to insure long periods of unattended operation with increased safety to equipment and experimental animals. (Dr. A. W. Pratt)

(4) Further modifications to commercial analog-to-digital converter-scanner-printer device to provide compatibility with ad-punch device of another manufacturer to provide common-language link to available electronic computer. (Dr. A. W. Pratt)

(5) Provided temporary electronic circuitry to make possible a determination that greater magnification and resolution was possible with the electron microscope than was provided by the manufacturer, and that such permanent modifications were desirable. (Dr. H. Kahler)

(6) Completed development and constructed electronic circuitry to insure flexible control, long-term stability and monitoring of input power to ultra-violet generators in U V germicidal device. (Dr. M. Elkind)

(7) Consulted with investigator concerning feasibility of electronic counting of colonies on glass dishes and examined equipment performing similar functions at other government installation. Recommended certain electro-mechanical aids to increase speed and reduce operator fatigue using existing optical method. These recommendations now being implemented. (Dr. M. Elkind)

(8) Presently consulting with investigator and instrument maker on equipment to be used in studying behavior patterns and conditioning of experimental animals when exposed to audible and shock stimuli. No construction under way at this time. (Dr. F. Smith)

(9) Presently conferring with investigator concerning electronic circuitry for electrophoresis apparatus. Certain circuit arrangement appear to be desirable to reduce cost and increase versatility of the equipment. No construction is yet under way. (Dr. F. Smith)

Significance to the program of the Cancer Institute: The ability to have these refinements to electronic devices makes it possible to make metabolic analyses on normal and tumor-bearing animals which heretofore were made indirectly.

Proposed course of project: To develop new and finer adaptations for the measurement of metabolic changes; for example, development of automatic computation of the large volume of available data. This will require the construction of new gas-sensing elements having proper electrical characteristics compatible with the operating requirements of existing analog computers.

Part B included No

Serial No. NCI-900
Laboratory of Physiology
Cancer Physiology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies in Tumor-Host Relationships

Principal Investigator: Julius White

Other Investigators: Florence Kate Millar and Jane Nicolet Fox

Man Years
Total: 8
Professional: 3
Other: 5

Project Description:

Objectives: To continue the study of the growth-stimulating factor(s) present in tumor tissue: A) To define the role of sodium as a dietary factor responsible for weight changes in tumor-bearing rats. B) To observe the influence of tumor tissue dietary factors on the changes in nitrogen utilization and the changes in electrolyte metabolism which occur during tumor growth.

Methods Employed: Sprague-Dawley rats bearing either the Walker 256 tumor subcutaneously or the Murphy-Sturm lymphosarcoma intramuscularly were maintained in metabolism cages on any one of the following diets: (a) a 20 percent casein control diet (C-20) adequate for optimal growth of normal rats; (b) a diet with lyophilized Walker tumor tissue replacing casein as a nitrogen source (T-24) (the sodium level of this diet was about 3 times that of the C-20 diet); (c) a 20 percent casein diet which contained either the sodium equivalence of the T-24 diet (C-20 + 0.9% NaCl) or twice the sodium equivalence (C-20 + 1.8% NaCl).

Food and water intake were measured and urine and feces collected daily. Urinary excretion of Na, K, Cl, phosphorus, total nitrogen, urea, NH_3, allantoin, and creatinine were measured. A procedure for analysis of Na and K in solid materials (diets, feces,

dried carcasses, and tumors) was developed involving a wet digestion with H_2SO_4 and HNO_3 and subsequent measurement of the Na and K in the digestion solution. For Cl content of diets, carcasses and tumors, the Schoniger catalytic combustion technique was used, followed by Cl measurement with a Cotlove titrator. Daily nitrogen, sodium, potassium, chloride and water balances were calculated, the water balance being based solely on the difference between water intake and urinary output. In a few experiments tumors were removed surgically during the course of the balance study. At the end of the balance study period (usually after 25-35 days of tumor growth), animals were sacrificed and carcasses and tumors lyophilized for analysis. In some cases the necrotic center of the Walker tumor was separated from the viable outer shell and the two portions analyzed separately.

Major Findings: The daily Na excretion of normal rats was dependent on the Na content of the diet ingested. Rats bearing the Walker tumor showed a drop in urinary Na beginning 15-20 days after tumor implantation even though the rats were in positive nitrogen balance. This drop in Na excretion occurred in rats ingesting any of the experimental diets. For example, the daily urinary Na of rats on the C-20 diet eventually fell to about 10 percent of the normal daily output; on diets containing more salt, urinary Na fell to 50 percent of normal. The drop in Na excretion represented an increased Na retention which was maintained throughout the remaining period of tumor growth. Chloride and water followed the Na pattern while K remained relatively unaffected.

Periodic single supplements of NaCl were given to normal rats on the C-20 diet and to the same rats after Walker 256 tumor implantation. During the normal period and the early stages of tumor growth (prior to 15 days), 80 to 100 percent of the dose was excreted. Twenty-five days or more after tumor implantation, salt "supplementation" resulted in almost complete retention (80-97% of the administered Na was retained).

The adrenals of rats bearing the Walker tumor and ingesting the C-20 diet showed marked hypertrophy when compared with adrenals from normal rats. This hypertrophy was characterized by enlargement of the zona glomerulosa. Adrenal hypertrophy was considerably lessened and the zona glomerulosa reduced in tumor-bearing rats ingesting either the T-24 or the C-20 + NaCl diet, presumably as a result of the increased level of Na in the diet.

Preliminary balance studies with rats bearing the Murphy-Sturm lymphosarcoma intramuscularly have been carried out to ascertain the roles of necrosis and tumor size in relation to Na retention. In rats bearing this fast-growing, relatively non-necrotic tumor and ingesting the C-20 diet, the daily urinary Na output fell to a minimal level after only 9 days of tumor growth, remained low for 5-7 days, and then rose to normal levels. The return to normal after 7 days of this Na excretion contrasted markedly with that of Walker tumor-bearing rats on the same diet. The weights of the lymphosarcomas at sacrifice were 31 to 42 grams. No hypertrophy of the adrenals was observed in animals bearing the lymphosarcoma.

The 1958 Annual Report described the gradual increase in allantoin excretion during tumor growth, the effect of diet on the allantoin excretion pattern of tumor-bearing rats, and the immediate reduction in allantoin excretion which followed surgical removal of the tumor. This study was extended to include the effect of tumor extirpation on Na excretion and allantoin excretion simultaneously. A rat bearing a Walker 256 tumor was fed the T-24 diet for 20 days until daily analysis showed Na retention and elevated allantoin excretion. Upon removal of a 35-gram tumor the Na and allantoin levels returned to normal within 72 hours. Six days after tumor extirpation the Na excretion began to decrease and the allantoin excretion to increase; a small mass was palpable at the original site. On the ninth day an 18-gram tumor was removed and again the Na and allantoin returned to normal.

Significance to the program of the Cancer Institute: The study of electrolyte changes in both tumor and host and the correlation of electrolyte metabolism with nitrogen utilization should yield information on the competition of the tumor and the host for available metabolites.

Proposed Course of Project: Studies of the growth-stimulating properties of tumor tissue will be extended to factor(s) other than the already identified Na, with special interest in their effects on the changes in nitrogen utilization and changes in electrolyte metabolism which occur during tumor growth.

Continuance of present studies will: (a) provide information on the role of tumor size in evoking and sustaining Na retention and adrenal hypertrophy, (b) enable us to ascertain whether the coincident Na and allantoin excretion effects during tumor growth may be related, and (c) show the carcass-tumor distribution of the Na retained by the tumor-bearing rat.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Millar, F. K., Brooks, R. H., and White, J.: Stimulation of appetite and growth in the tumor-bearing rat by a variety of tissues. J. Nat. Cancer Inst. 22: 685-696, 1959.

White, J., Toal, J. H., Millar, F. K., and Brooks, R. H.: Walker carcinosarcoma 256 tissue as a dietary constituent. III. Sodium as a factor in stimulation of water and food intake and growth in tumor-bearing rats. (Accepted for publication; to appear in J. Nat. Cancer Inst., Jan. 1960.)

Serial No. NCI-905
Laboratory of Physiology
Cancer Physiology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Iodide Concentrating Mechanism of the Thyroid Gland

Principal Investigator: Seymour H. Wollman

Man Years (calendar year 1959):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To learn about (a) the mechanism by which the thyroid gland maintains a concentration of iodide elevated above that in the blood, and (b) the mechanism by which the thyroid gland accumulates protein-bound iodine.

Major Findings: This year most of our work on this project has been theoretical. We have set up simple models of the thyroid gland and obtained from them a quantitative understanding of rates of binding of radioiodine, rates of transport of radioiodide into and out of the thyroid, rates of equilibration of radioiodide between the thyroid gland and blood, and of the clearance curves. These models are an extension of earlier published models, where organic binding is blocked, to the case where binding is permitted. Agreement between the simple models and experimental data is remarkably good except in the case of data from hypophysectomized mice. This preparation requires a more complex model with internal heterogeneity.

Significance to the program of the Cancer Institute: This work is leading to an understanding of the relative importance of various factors in controlling the accumulation of radioisotopes by tissues.

Proposed Course of Project: This project will be continued with somewhat more emphasis on investigation of the role of blood flow to the thyroid in the accumulation of radioiodine by the thyroid gland.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Wollman, S. H., and Reed, F. E.: Transport of radioiodide between the thyroid gland and blood in mice and rats. Am. J. Physiol. 196: 113-120, 1959.

Wollman, S. H.: Concentration and organic binding of radioiodide by the thyroid gland. Ann. N. Y. Acad. Sci. In press.

Honors and Awards relating to this project:

Invited lecture: "Concentration and Organic Binding of Radioiodide by the Thyroid Gland." Presented at the Symposium on Modern Concepts of Thyroid Physiology held by the New York Academy of Sciences on November 5-7, 1959.

Serial No. NCI-906
Laboratory of Physiology
Cancer Physiology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Properties of Transplantable Thyroid Tumors

Principal Investigator: Seymour H. Wollman

Other Investigators: Dr. Edward B. Price, LP NCI
Dr. Leonard Warren, LPT NIAMD
Dr. Samuel S. Spicer, LPH NIAMD

Cooperating Units: NCI, Laboratory of Pathology -
Dr. Edward B. Price (Serial No. NCI-518)
NIAMD, Laboratory of Pharmacology and Toxicology -
Dr. Leonard Warren (Project Serial No. not available)
NIAMD, Laboratory of Pathology and Histochemistry -
Dr. Samuel S. Spicer (Project Serial No. not available)

Related Project: The Clinical Endocrinology Branch of NIAMD is studying some of the transplantable thyroid tumors.

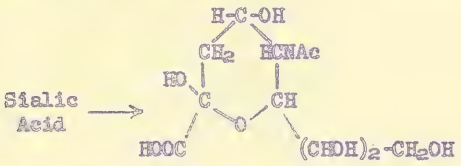
Man Years: (calendar year 1959)
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To study properties of transplantable thyroid tumors in the rat: Variety of histologic types and their progression, correlation of histology with biochemistry and physiology of the tumors.

Major Findings: Some of the lines of transplantable tumors of the thyroid gland which we have been studying contain a good deal of intracellular periodic acid - Schiff positive material. Homogenates of

these tumors are very viscous and this suggested the presence of mucins. Other lines contained eosin negative colloid. An attempt was therefore made to characterize the mucins in almost all our lines of tumors including those with eosin negative colloid. Many of the lines contained acid mucins in the colloid as indicated by staining by basic dyes. The acid group was shown to be sialic acid and not sulfate by a variety of histochemical procedures including the dependence of stainability on pH and by removal of stainability by a specific sialidase preparation.



In rats carrying one of the tumors with intracellular acid mucins, the sialic acid level was elevated in serum and urine.

Some of the more functionally active thyroid tumors contain neutral mucins as does the thyroid. In an autoradiographic study it was observed that in a tumor with neutral mucins all follicles formed organic iodine, whereas in a tumor with acid mucins in the colloid some follicles were non-functional. However, the relation of acid mucins to thyroid function is unclear as yet.

Significance to the program of the Cancer Institute: These tumors are valuable because of the variety of their easily identified histologic and biochemical characteristics. Physiologic studies will give insights into the correlation of structure and function.

Proposed Course of Project: Studies of the physiology of these tumors will be continued.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Honors and Awards relating to this project:

Invited Lecture: "Progression in Transplantable Thyroid Tumors Arising in Rats Fed Thiouracil." Presented at the Symposium on Conditioning Factors in Neoplasia held at the annual meeting of the American Association of Pathologists and Bacteriologists in Boston, April 23-25, 1959.

Serial No. NCI-910
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Tumor Autoradiography

Principal Investigator: James C. Reid

Other Investigator: Julius White

Man Years (calendar year 1959):
Total: None - Completed project
Professional:
Other:

Project Description: As stated in the last annual report this project was completed in 1958. It is listed again this year for the purpose of reporting a publication resulting from the project. See Part B.

Part B included Yes

Serial No. 910

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Reid, James C., and White, Julius: An autoradiographic study of tumor dynamics. J. Nat. Cancer Inst. 22: 845-867, 1959.

Serial No. NCI-919
Laboratory of Physiology
Cancer Physiology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigation of Urinary Excretion of Nucleotides and their Congeners in Leukemia

Principal Investigator: James C. Reid

Cooperating Units: General Medicine Branch, NCI-4-719 (C)

Man Years (calendar year 1959):
Total: 2
Professional: 2/3
Other: 1-1/3

Project Description:

Objectives: The purpose of this project is to determine whether there is a detectable urinary excretion of nucleotides or nucleosides, and, if so, to develop an analytical method for their identification and quantitative estimation. The method is also to be applicable to free purines and pyrimidines. The technique will be used to investigate the metabolism of these substances in leukemic patients.

Methods Employed: The methods used are primarily chromatographic.

Major Findings: As was stated in the last report, technical difficulties cropped up when the analytical procedure developed earlier was applied to urine. The correction of these difficulties has proved more troublesome than was expected, new problems tending to arise as old ones are disposed of. These range in character from practical ones, such as lyophilization losses, to chemical ones, such as the effect of variation of loading and washing volumes upon the sharpness of group separations in the preliminary stripping columns.

These difficulties have gradually been resolved although xanthosine recovery is still somewhat low and there remains a tendency to non-reproducibility in the performance of the ammonium acetate gradient system used for the analysis of the major nucleoside fraction. Both of these effects are probably related to

instability of the pH in certain regions of the gradient. It is anticipated that the cause of this can be discovered and controlled, but so far it has stood in the way of our publishing the procedure. Insofar as the analysis itself is concerned, however, these items are not critical ones and urinary analysis has therefore been resumed with the running of a normal control, which is in progress at the moment and appears to be going smoothly. The collection of leukemic urines has been going on steadily and a number of specimens are on hand.

With chromatographic problems apparently under control, serious consideration has been devoted to the remaining major problem to be dealt with, namely quantitative estimation of the individual components of the mixed fractions obtained in the urinary chromatograms. Although it was always reasonably certain that this could be accomplished, in crude fashion at least, by paper chromatography, the complexity encountered in the urinary patterns made it apparent that this method would gravely complicate an already unwieldy procedure.

As an alternative, the possibility of employing differential spectrophotometry has been investigated. It was soon found that a limited treatment based on the selection of n points from the ultraviolet spectrum of a mixture of n components does not give acceptable accuracy even for simple mixtures. Application of more powerful mathematical curve-fitting techniques, however, gives such excellent results in preliminary trials that a thorough test of the possibilities of this approach is underway. This requires the aid of an electronic computer. A program for the IBM-650 has been written by Dr. Arnold Pratt, Laboratory of Physiology, and is under test.

Significance to the program of the Cancer Institute: The ultimate aim of this project is the accumulation of information concerning disturbances of nucleic acid metabolism in leukemia and the mechanism of the therapeutic action of nucleotide-related analogues as well as the mechanism of their toxic side effects.

Proposed Course of Project:

1) The development of a standardized analytical procedure based on differential spectrophotometry will be a major activity for the immediate future.

2) The mass of numerical data being generated requires some instrumentation for data reduction not only to save time but to avoid errors. This will involve digitalization and automatic punching of data from the Cary spectrophotometer and automatic sample handling equipment. Additional lyophilization equipment must also be built.

3) Chromatography of urine specimens can proceed while 1) and 2) are going on and it is expected that a survey of the acute leukemic group will be achieved this year.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Loo, T. L., Michael, M. E., Garceau, A., and Reid, J. C.:
6-Thiouric acid, a metabolite of 6-mercaptopurine.
J. Am. Chem. Soc. 81: 3039-3041, 1959.

Serial No. NCI-920
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Identification of Compound A-20

Principal Investigator: James C. Reid

Man Years (calendar year 1959)
Total: 1-1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: Identification of what is believed to be a new amino acid found in rat liver.

Methods Employed: Chromatographic methods are used for isolation of the substance. The methods of physical and organic chemistry are used to accumulate information which will permit an identification.

Major Findings: Activities on this project have been devoted to organic chemistry in an attempt to develop both a synthetic procedure for the compound and degradative procedures for converting it to known substances. These have so far been unsuccessful but work is continuing.

Significance to the program of the Cancer Institute: The substance being studied is presumably related to protein metabolism which has basic importance in tumor growth.

Proposed Course of Project: Evaluation of synthetic and degradative schemes in progress will continue.

Part B included No

Serial No. NCI-925
Laboratory of Physiology
Cancer Physiology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Protein Synthesis and its Antagonism in Normal and Cancer Cells

Principal Investigator: Marco Rabinovitz

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

a. A comparison of the mechanisms for the formation of protein in normal and cancer cells.

b. An evaluation of the structural requirements for antimetabolite activity of amino acid analogues on the protein synthetic mechanism of mammalian cells, and an elucidation of the biochemical lesions produced.

c. Extension of the above two objectives to the tumor-bearing mouse and rat with the view of selective inhibition of protein synthesis in cancer cells.

Methods Employed:

a. Organic synthetic techniques are developed for preparation of potential amino acid antagonists.

b. Incorporation of carbon-14 labeled amino acids into protein of surviving cells is measured. Such studies involve radioactive counting techniques, chromatography, and colorimetry.

c. Subcellular fractions (nuclei, mitochondria, microsomes, soluble proteins) are isolated by differential centrifugation from cancer and normal cells which had been treated either in vitro or in vivo with amino acid antagonists and C-14 labeled amino acids. The kinetics of the block in amino acid utilization by these fractions are evaluated.

d. Tumor-bearing animals are placed on various dietary regimens and the effects of amino acid antagonism on tumor growth and protein synthesis are determined.

Major Findings: Several amino acid analogues which contain a sulfur atom in place of a methylene group of the side chain have been synthesized. These include 4-thia-L-glutamine, 4-thia-D-glutamine and 4-thia-L-homoglutamine. Two new compounds, 4-thiamethionine and 4-thiaisoleucine, have been prepared, and an improved synthesis for 4-thialysine was developed. Several other analogues, such as α-amino-β-chlorobutyric acid (valine antagonist) and O-methylthreonine (isoleucine antagonist) were synthesized.

We have previously shown that methionine sulfoximine reduces by half the protein synthetic rate of washed Ehrlich ascites cells because it prevents the biosynthesis of the essential amino acid, glutamine. This inhibition has now been shown to be shared by the nuclear and cytoplasmic protein-forming systems.

The mixed disulfide of methyl mercaptan and cysteine, which may be called 4-thiamethionine, inhibits protein synthesis by Ehrlich ascites cells at low concentrations. This inhibition is not prevented by methionine but is by glutathione. The analogue is non-toxic to mice.

Certain amino acid analogues which replace their metabolites in the early stages of protein synthesis will later block the protein-synthetic mechanism. This biochemical lesion can be magnified by the use of several amino acid antagonists. The inhibition is only partially prevented in the presence of the corresponding natural amino acids. The cause of this phenomenon is being investigated.

A potent lysine antagonist in the Ehrlich ascites cell, 4-thialysine, is well tolerated by mice on a lysine-deficient diet. In vivo experiments indicate that the antagonist will interfere with lysine-C^{14} incorporation in tumor and liver, but not in [illegible]

Significance to the program of the Cancer Institute: These studies utilize the action of amino acid antimetabolites as a means of comparing protein anabolism in normal and cancer cells. In addition, structure-activity relationship for producing antagonism and studies on mechanism of inhibition, both in vitro and in vivo, should furnish basic data of potential use for selective inhibition of cell types and specific protein-forming systems.

Proposed Course of Project: All aspects of this project will be continued.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Rabinovitz, M., and Tuve, R. L.: Antimetabolite activity of lysine analogues on lysine incorporation into rat bone marrow protein in vitro. Proc. Soc. Exp. Biol. Med. 100: 222-224, 1959.

Serial No. NCI-927
Laboratory of Physiology
Cancer Physiology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Sialic Acid in the Thyroid Gland

Principal Investigators: Seymour H. Wollman
Dr. Leonard Warren, NIAMD

Cooperating Unit: NIAMD, Laboratory of Pharmacology and Toxicology
Dr. Leonard Warren (Project Serial No. not available)

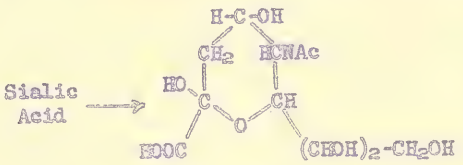
Related Project: Dr. Robert W. Bates, Section of Endocrinology, Laboratory of Nutrition and Endocrinology, NIAMD

Man Years (calendar year 1959)
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To study how the concentration of sialic acid in the thyroid gland varies with physiologic state of the gland.

Major Findings: The finding that sialic acid, a nine-carbon amino sugar derivative is a constituent of the colloid in thyroid tumors led us to examine



normal thyroid glands for sialic acid. We obtained a pure preparation of thyroglobulin from Dr. Edelhoch and found that it contained one per cent sialic acid. The sialic acid concentration in the thyroid gland was highest in glands from hypophysectomized rats, less for normals, and least for rats fed thiouracil. The sialic acid concentration in the thyroid began to decrease after feeding thiouracil for more than 24 hours and reached a minimum by about 9 days. It is not surprising that the sialic acid concentration was decreased since thyroglobulin is resorbed in the thyroid glands of thiouracil fed rats. Since it is believed that thyroglobulin is proteolysed before thyroxine is liberated, we investigated whether a product of proteolysis namely free sialic acid could be found in the thyroid gland. The concentration of free sialic acid in the thyroid was lower in hypophysectomized rats than in normals. In rats fed thiouracil, the concentration of free sialic acid in the thyroids was highest when the concentration of total sialic acid was decreasing most rapidly (after approximately 2 days of thiouracil feeding).

Significance to the program of the Cancer Institute: Sialic acid is a prominent component of some transplantable thyroid tumors. Studies on sialic acid in the normal gland were desirable to get insights into reasons for the presence of the sialic acid in tumors.

Proposed Course of Project: This project will be completed after a few additional studies.

Part B included No

Serial No. NCI-911
Laboratory of Physiology
Energy Metabolism Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Total Metabolism

Principal Investigator: A. W. Pratt

Other Investigators: W. C. White and B. E. Burr

Man Years (calendar year 1959):
Total: 5-2/3
Professional: 3
Other: 2-2/3

Project Description:

Objectives: The study of the total metabolism and, particularly, the total energy metabolism of normal and tumor-bearing animals.

Methods Employed: The studies utilize, in addition to routine chemical and physical determinations, the techniques of analytical calorimetry as developed in this laboratory (Serial No. NCI-911, ...7] and 1958).

Major Findings: Energy metabolism studies measure growth of an animal as accretions of nitrogen, calories, water and change in the animal's total mass. It is necessary, therefore, to express an animal's growth and change in body composition in terms of these variables.

A systematic study of several nitrogen-calorie relations was initiated during the previous reporting period and has been continued during this reporting period. We have determined that the dry total carcass of a sacrificed animal can be described in these terms by its fat and non-fat content. It was found, too, that the nitrogen and calorie content of these two body compartments remained constant relative to Kcal and nitrogen content among the animals over 150 to 300 gram body weight range. It was assumed that a dry, total carcass could be considered to be a mixture of

these two entities, and that the proportions of fat and non-fat in an individual carcass could be described by a straight-line function on a plot of mgm N/gm and Kcal/gm of dry, total carcass (NCI-911, 1958).

The equation of the theoretical line was

$$\text{mgm N/gm} = -29.4 \text{ Kcal/gm} + 274 \qquad (1)$$

As a test of the theoretical line, the dry total carcasses from 30 animals having body weights ranging from 150-300 grams and also having been maintained on various diets were examined for nitrogen and calorie content. Using the principle of least squares, a straight line was fitted to the data points. The equation of the fitted line was

$$\text{mgm N/gm} = -29.2 \text{ Kcal/gm} + 273 \qquad (2)$$

The good agreement between equations 1 and 2 is apparent. Variance of an animal about the theoretical line was 3.6149 $(\text{mgm N/gm})^2$ and the standard deviation was 1.9 mgm N/gm. The 95% confidence limit was approximately two standard deviations. Further analysis reveals that the variation about the theoretical line is primarily animal variation. In fitting the experimental line, it was assumed that the value for Kcal/gm was given and the value of mgm N/gm was varying. Both variables, however, can vary in this system; therefore, an extensive bivariant analysis is being carried out in an effort to establish the expected normal variation within rather precise limits. In this way the nitrogen-calorie relation will give a measure of gross body composition and also an indication of change in elemental composition of the fat or non-fat components. This would be indicated, of course, by experimental points which fell outside the zone of normal variation.

With the development of this mathematical technique, it appears profitable to study the change in gross body composition and, if present, change in elemental composition of the carcass of the tumor-bearing animal. Such experiments are now in progress.

The available data, though insufficient, indicate that the carcass of the tumor-bearing animal undergoes severe gross compositional changes during tumor growth but that no discernible change in elemental composition occurs. This is a point of considerable importance: first, in understanding the general metabolism of the

tumor-bearing animal and, additionally, in the construction of a mathematical model from which one can predict, solely from the energy metabolism data, the tumor growth and the change in carcass weight and composition. To this end, the derivation of a satisfactory mathematical model based on the nitrogen-calorie, weight change relations is our most important objective.

Measurement of the animal's total energy metabolism involves both indirect and direct calorimetric techniques. It would appear that we have achieved a routine operation of the direct calorimeter with controls that compensate for wide variations in the temperature of the water supply and for moderate variations in room temperature. A routine study involves four to six days of continuous animal observation divided into two-or three-day periods. The equipment has been modified to record data on punched paper tape, which when converted to punched cards can be processed in the M.I.T. computer (IBM-650). The computer program has been written by our group and thus allows for the necessary flexibility for processing the data.

The present experiments are designed to measure heat production and heat loss, thus providing a measure of calorie balance in the tumor-bearing animal. A prime objective is to determine the agreement between the indirect and direct calorimetry of the tumor-bearing animal. At present we are beset with changing electrical characteristics of the carbon dioxide transducer. We are unable to compensate for these electrical changes and have been forced to undertake re-instrumentation of the carbon dioxide transducer. A considerable amount of progress has been made.

During this reporting period both the routine analytical and isotope mass spectrometry have been consolidated under Mr. Burr. The majority of the work in this area relates to the routine activities of the laboratory. However, a number of pilot research projects have been completed in an effort to better utilize the analytical instrument in our physiological investigations. One such project which will be pursued further is the measure of an oxygen balance in hopes that an independent measure of metabolic water can be achieved. Additionally, Mr. Burr is collaborating with members of the Radiation Branch in a study of the photochemical reactions of hydrogen atoms in radiochemical reactions of organic compounds in aqueous solutions (NCI-697).

Significance to the program of the Cancer Institute: Studies of the total energy metabolism offers the single precise means of determining an animal's general metabolic response to disease. Interpretation of the total energy metabolism data as a change in the physiologic state of the animal will add immeasurably to the definition of the disease state.

Proposed Course of Project: To continue the evaluation of nitrogen-calorie relationships in relation to body composition and total energy metabolism in the normal and tumor-bearing animal.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Pratt, A. W., and Putney, F. K.: Gross body composition and total energy metabolism in normal rats. Am. J. Physiol. 197: 660-666, 1959.

Serial No. NCI-914
Laboratory of Physiology
Physical Biology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: In Vivo Measurement of Tumor pH with the Capillary Glass Electrode

Principal Investigator: H. Kahler

Man Years (calendar year 1959):
Total: 2
Professional: 1/3
Other: 1-2/3

Project Description:

Objectives: The object of this project is to determine the in vivo pH of tumors under different experimental conditions.

Methods Employed: Fine-pointed glass electrodes, selected after passing rigorous tests, were inserted into tumors in the leg muscle of rats and the pH recorded for several hours. At the end of the first hour, glucose was injected intravenously or intraperitoneally, and the effect of this upon the pH observed. At autopsy, the position of each electrode was determined with respect to the gross anatomical features of the tumor.

Major Findings: In contrast to previous work in which the glucose was injected intraperitoneally, the intravenous injection produces a different blood condition. The intraperitoneal glucose causes fluid to flow from the blood stream into the peritoneal cavity and hence causes a dessication of the extra-peritoneal body. On the other hand, the intravenous glucose injection causes an increased blood volume which is repeatedly seen to enhance the blood volume in tumors. On our pH diagrams the intravenous injection causes a more rapid drop in pH and a more rapid return to normal levels.

The Novikoff Hepatoma which becomes necrotic was used to test the pH drop of necrotic versus solid tumor tissue after intravenous injection of glucose. It was found that the solid tumor tissue gives several times the pH drop of the necrotic tissue. In 12 necrotic and 25 non-necrotic tumors the pH drops were 0.16 pH and 0.65 pH units, respectively.

Significance to the program of the Cancer Institute: The pH of tumor tissue is essential to an understanding of chemical processes in the tumor.

Proposed Course of Project: It is expected that this project will be concluded during the next year.

Part B included No

Serial No. NCI-915
Laboratory of Physiology
Physical Biology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cellular Particulates

Principal Investigator: H. Kahler

Other Investigator: Bolivar J. Lloyd, Jr.

Cooperating Units: NCI, Laboratory of Biology, Leukemia Studies Section - Dr. L. W. Law (Serial No. NCI-427)
NCI, Laboratory of Pathology - Dr. C. J. Dawe (Serial No. NCI-521a)
NIAID, Laboratory of Infectious Diseases - Dr. W. P. Rowe (Project Serial No. not available)

Man Years (calendar year 1959):
Total: 2-1/3
Professional: 2/3
Other: 1-2/3

Project Description:

Objectives: Parotid Tumor Agent or Polyoma Virus -- The relation between concentration of particles as determined by electron microscope technics and various biological effects.

Methods Employed: Partially purified virus was sedimented on to collodion films mounted in ultracentrifuge cells. The concentration of the virus was then determined by counting electron microscopic images of the virus. Dilutions were then injected into newborn mice for tumor induction assays. Aliquots were also used for cytopathogenic effects, HA and HI titer.

It is not possible to report on the results of this work for another 6 months.

Major Findings: Experiments started 1-1/2 years ago with Drs. Stewart and Eddy were concluded. These preliminary tests were to find in what size class their polyoma virus was. Tissue culture fluids were centrifuged in density gradients, and fractions taken from the ultracentrifuge tubes were injected into newborn mice. At death these were autopsied by Dr. Stanton for tumor occurrence. The conclusion reached from these experiments was that the size of the virus particles was consistent, within large error limits, with the values found later from electron microscopy.

Preliminary tests on Moloney's Leukemia virus obtained from mouse spleens showed that there was a similarity of particles in the mouse tumor and the normal spleen control. Methods for differentiating between normal and virus particles must be utilized.

Cell-free filtrates of Murphy-Sturm Lymphosarcoma extracts were found to induce bony growths in young rats which could be propagated by cell-free extracts of the growths. It turned out that these effects were caused by pleuro-pneumonia-like organisms (PPLO) which seem to grow well in certain tumors. No evidence was obtained to implicate a virus in the propagation of this tumor.

Significance to the program of the Cancer Institute: Provides evidence bearing on the virus concept of some cancers.

Proposed Course of Project: Continuation of the same type of work on other virus strains, as well as further detailed study of the same virus.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Kahler, H., Rowe, W. P., Lloyd, B. J., and Hartley, J. W.: Electron microscopy of mouse parotid tumor (Polyoma) virus. J. Nat. Cancer Inst. 22: 647-657, 1959.

Gavin, M. A., and Lloyd, B. J.: Knives of high silica content glass for thin-sectioning. J. Biophys. and Biochem. Cytology 5: 507 (only), 1959.

Serial No. NCI-916
Laboratory of Physiology
Physical Biology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Characterization and Comparison of Nucleic Acids, Nucleoproteins, Nucleases, and Nuclease Inhibitors of Normal and Malignant Tissues and of Body Fluids

Principal Investigator: Joseph Shack

Man Years (calendar year 1959):
Total: 1-1/2
Professional: 1/2
Other: 1

Project Description:

Objectives:

a) To determine whether the nucleic acids and nucleoproteins of normal and malignant tissues differ in physical, chemical or biological properties.

b) To determine the nature and amount of nucleases and nuclease inhibitors present in tissues and body fluids.

c) To develop new procedures for the study and characterization of nucleic acids.

Methods Employed:

a) Procedures for isolation of nucleic acids and nucleoproteins; controlled modification by heat, acid, alkali, enzymes, etc; study of these by various chemical and physico-chemical procedures.

b) Various procedures for preparation of extracts of tissues and body fluids suitable for assay of enzyme or enzyme inhibitor.

Major Findings:

a) Further investigation of the interaction of deoxyribonucleate and magnesium ions indicated that

1) the binding is essentially independent of pH in the range where no titration of the nucleate occurs, and
2) the quantity of Mg bound is strictly proportional to the concentration of DNA at a given free Mg ion concentration.

b) Trypsin destroys the DNase I inhibitor derived from human white blood cells both in the absence and in the presence of DNase I, but does not affect the DNase I. This destructive action of trypsin can be prevented by the presence of soybean trypsin inhibitor. The destruction of DNase I inhibitor by a factor present in human serum is, by contrast, not prevented by this soybean inhibitor. These findings have provided the basis for methods of assaying DNase I inhibitor in the presence of a relative excess of DNase I and of assaying DNase I when inhibitor is present initially. Use of these procedures have shown that normal human urine, which contains large amounts of DNase I, contains no soluble DNase I inhibitors.

Significance to the program of the Cancer Institute: The significance of research on nucleic acids and nucleoproteins in connection with the cancer problem is related to the key role of these substances in genetic processes, bacterial and viral transformations, virus reproduction, protein synthesis. Interest in nuclease and nuclease inhibitors relates to the necessity of controlling DNase action during isolation of DNA, particularly if they are to be tested for biological activity. In addition, it is possible that the amount and nature of the enzymes and enzyme inhibitors may be in some way related to the growth of tumors present in the host.

Proposed Course of Project:

a) Further comparison of nucleic acids and nucleoproteins derived from normal acid malignant tissues.

b) Further studies of the nucleases and nuclease inhibitors of the body fluids of normal humans, cancer patients, and appropriate experimental animals.

In view of the rather specific association of high DNase I inhibitor content with the white cells of blood, it is contemplated that considerable attention will be given to studies of this substance, particularly in relation to various hematological diseases.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Shack, J., and Bynum, B. S.: Determination of the interaction of deoxyribonuclease and magnesium ions by means of a metal ion indicator. Nature 184: 635-636, 1959.

Shack, J.: The influence of sodium and magnesium ions on the action of deoxyribonuclease II. J. Biol. Chem. 234: 3003-3006, 1959.

Serial No. NCI-926
Laboratory of Physiology
Physical Biology Section
Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Purification and Characterization of the Myxoma Virus Transforming Agent

Principal Investigators: Joseph Shack and Lawrence Kilham, Division of Biological Standards (Project Serial No. is not available)

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2
Other: 0

Project Description

Objectives: Work during this year was concerned with both transforming agent prepared from myxoma virus a) by heat alone (HAM) and b) by a combined heat-urea treatment (UREA-HAM). A major objective was to purify these to a state suitable for study by methods of chemistry and electron microscopy as well as in terms of their transforming potency.

Methods Employed: Procedures for preparation of transforming agents and determination of their susceptibility to various enzymes. Numerous methods used in preparation of viruses and viral products, e.g. differential sedimentation, genetron treatment, etc.

Major Findings:

a) Attempts at substantial purification of myxoma virus as well as of transforming agents from extracts of tumors were without success. Later work involving myxoma virus grown in tissue culture was, however, very promising in this respect. It was found that active transforming agents could be prepared from the tissue cultures as well as from extracts of tumors.

b) Attempts to isolate an active separated (deproteinized) nucleic acid by the phenol procedures were unsuccessful.

c) In addition to differences in susceptibility of TAM and UREA-TAM to deoxyribonuclease (reported last year), significant differences were found in the behavior in different types of tissue culture. TAM is effective in a variety of cultures such as squirrel and monkey kidney, rat embryo, and rabbit kidney (either domestic or cottontail). UREA-TAM was active only in the kidney cultures derived from rabbits, the natural host of both fibroma and myxoma viruses. It was found that both TAM and UREA-TAM can enter the cells alone (in absence of fibroma virus); however, any UREA-TAM that enters is destroyed in a relatively short time (15 minutes to 3 hours in different experiments), whereas TAM remains undestroyed for as long as 3 days.

Significance to the Program of the Cancer Institute: The significance is related to the role of viruses in cancer etiology.

Proposed Course of Project: This project was ended in September 1959 as a result of the termination of Dr. Kilham's virology program. It is hoped that the techniques and approaches developed in the course of this project will find application in other projects now under consideration.

Part B included Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Shack, J., and Kilham, L.: Relation of myxoma deoxyribonucleic acid (DNA) to fibroma-myxoma virus transformation. Proc. Soc. Exp. Biol. Med. 100: 726-729, 1959.

Serial No. NCI-688
1. Radiation Branch
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Development, Fabrication and Maintenance of Specialized Equipment for Radiation Research

Principal Investigator:

Calvin R. Mencken

Other Investigators:

Edward J. LeBrun and Ralph C. Whittaker

Cooperating Units:

None

Man Years (calendar year 1959)
Total: 3
Professional: 2
Other: 1

Project Description:

Objectives: To design and fabricate laboratory instruments and devices that are not available on the open market, and/or incorporate refinements to said devices to make them applicable for the unusual services that are required in radiation research. To service or assist the investigators in servicing such equipment as the Van de Graaff generators and fabricate special parts for same, as requested by said investigators. To service less significant equipment as requested. The accompanying list of projects is condensed and does not give a complete or wholly accurate description of every task involved. The more significant ones will be designated by an asterick (*). If more complete and accurate information is desired, it is suggested that the investigator whose name accompanys the item be consulted.

It will be noted that a part of the list is involving Glass Blowing. This will be listed separately and will be designated as such.

Project Description: (Continued)

Major Projects: Radiation Branch

Dr. Howard Andrews:
Fabricate miscellaneous parts for, and assist in repairing and servicing 3 Mev Van Der Graaff.
Rebuild rotating device for 3 Mev.
Modify TV camera support.

Mrs. Peterson:
Design and fabricate, pivoted mouse cage to determine radiation response.

Mr. Murphy:
Fabricate ionization probes as requested.

Mr. Robert Swain:
Design and fabricate device to motivate ionization chambers by remoted control.
Fabricate Isodose Scanning device.
Fabricate ionization chambers for continuous monitoring of 2 Mev x-ray beam.
Design and fabricate boring device to machine the cone of the 2 Mev.
Service to 2 Mev. Lifting mechanism.

Dr. M. Elkind:
Build UV radiation apparatus for cellular work.
Fabricate fixture for maintaining control temperature of mammalian cells during radiation.
Convert lantern slide projector for the purpose of examining fixed and stained colonies on petri dishes.
Fabricate gas fixture for petri dishes during phase microscopy.
Microscope marking device for marking cones on petri dishes.

Dr. S. Sneider:
Design and fabricate skull clamp for radiation treatment of the head and neck.
Fabricate treatment wedges as per specifications.

Dr. H. Suit:
Development of needles for interstitial radiation therapy with cobalt and irididium wire.

Dr. K. Brace:
Develop adjustable tubular gauges for measuring treatment distances.
Design and fabricate remote hydraulic control for [illegible] camera.

Project Description: (Continued)

Dr. P. Riesz:
Fabricate holder for syringe radiation, including a monitoring ionization chamber to be used on 250 KVP x-ray machine.

Dr. Maxwell:
Modify recorder to pull paper chromatographs across gas flow counter at same speed as chart.
Fabricate hot air heater bath for low temperature evaporation of corrosive liquids.

Dr. Falconer Smith:
Fabricate agglutination test rack.
Design and fabricate automatic drain for colorimeter.

Miss H. Gump:
Adapt dissecting microscopes and investigating microscops to be used in technique for making window transplants.
Also associated tooling required for window splints, etc.

Glass Blowing:
* Develop and frabricate special petri plates so designed to minimize shadow effects in ultra violet experiments.
* Fabricate special tissue culture tubes with optically flat sides.

Dr. Elkind:
* Fabricate modified type T-60 tissue culture flasks.
Fabricate glass cylinders.
Repairs on vacuum apparatus.

Dr. Maxwell:
Fabricate chromatography columns.
Repairs on water still.
Fabricate condensors.

Dr. Falconer Smith:
* Fabricate tubes with optical bottom for photographic study.

Dr. Howard Andrews:
Fabricate glass cylinders for use with 3 Mev.
* Fabricate electrode assembly for radiation purposes.

Dr. P. Reisz:
* Fabricate degassing apparatus used in radiation experiments.
* Fabricate quartz distillation apparatus.
Fabricate glass discs.

Dr. Robinson:
Fabricate atomizers.
Fabricate special type drying tubes.

Dr. Rose:
Modify Erlenmeyer flasks, containing internal well.

Department of Physiology

Dr. Pratt:
Modified thermal conductivity cell block with dual construction.
Fabricate lucite humidity cells.

Dr. F. K. Millar:
Specially constructed aluminum support for lyophilizer.

Dr. M. Rabinovitz:
Stainless steel drying racks for chromatography paper.

Dr. Kahler:
Modifications to ultracentrifuge rotor.

B. E. Burr:
Miscellaneous adaptations and modifications for mass spectrometer.

Dr. Reid:
Glass Blowing:
* Fabricate glassware for modified lyophilizing apparatus using glass pipe.
Fabricate chromatography columns.

Dr. Pratt:
Glass Blowing:
Fabricate monometer with tungsten electrodes.
Repair glassware.

Dr. Rabinovitz:
Glass Blowing:
Modify Warburg flasks.

Dr. White:
Glass Blowing:
* Fabricate lyophilizing apparatus.
fabricate all glass separators.

Mr. Burr:
* Fabricate gas sampling tubes for use in mass spectrometer.
Fabricate nitrogen condensor for same.
* Fabricate control valve for liquid nitrogen automatic fill.

Dr. Kahler:
* Fabricate all glass electrodes.

Part B included Yes ☐ No ☒

Serial No. NCI-642
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Antibody Production in Irradiated Mice: Effect of Chronic and Intermittent Exposure to Ionising Radiation.

Principal Investigator:

Falconer Smith

Other Investigators:

Marie M. Grenan, John Owens

Cooperating Units:

None

Man Years (calendar year 1959)

Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: To determine the effects of chronic gamma radiation exposure on antibody formation and to observe details of time-dose relationships in split radiation exposures as measured by the capacity of mice to form specific antibody.

Methods Employed: 1) Exposure of mice to cobalt^{60} source is being arranged. At present the calibration of the source is under way. 2) Exposure of mice to a standard x-ray source is being carried on.

Major Findings: 1) Recovery of immune responses after two doses of x-rays given at varying intervals of time demonstrate a relationship apparently comparable to observations made in studies of lethality associated with split x-ray exposures. 2) Multiple radiation exposures are cumulative in their injurious effect on the immune response in mice although the injurious effect is not apparent in so far as circulating white blood cell count is concerned.

Significance to cancer research: Studies attempting to clarify the nature of chronic and multiple radiation injury to processes of protein synthesis is germane to standard radiation therapy procedures.

Proposed course of project: Chronic exposure studies with mice of various ages, using the newly developed Co^{60} source are to begin. X-ray exposure studies with divided doses are nearing completion. Chronic exposures of splenectomized mice will be undertaken in order to determine the role of the spleen in antibody formation in such preparations.

Part B included Yes ☒ No ☐

Serial No. NCI-642

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B.

Honors, Awards, and Publications:

Publications other than abstracts from this project:

Hemolysin formation in mice following partial, total splenectomy of spleen transplantation. Proc. Soc. 101: 363, 1959.

Honors and Awards relating to this project:

None

Serial No. NCI-659
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Action of Endotoxin in Irradiated Animals: Reduced Mortality; Increased Resistance to Infection; Advanced Hematopoietic Recovery.

Principal Investigator:

Willie W. Smith, Ph. D.

Other Investigators:

Ilo M. Alderman and Ruth G. Simpson

Cooperating Units:

None

Man Years (calendar year 1959):

Total: 4/5
Professional: 1/5
Other: 3/5

Project Description:

Objectives: To determine the effects of bacterial endotoxins on the irradiated animal and to discover the physiological changes responsible for such effects.

Methods Employed: Endotoxins are injected before or after irradiation and effects noted on: (a) mortality; (b) resistance to experimental infection; (c) hematopoietic recovery.

Major Findings: In addition to earlier findings we have learned that (a) endotoxin, unlike colchicine, is effective in quite young animals, (b) endotoxin, unlike colchicine is effective in guinea pigs and (c) unlike the action of colchicine in mice, hamsters and rats, endotoxin is effective when given after, but not before, irradiation.

Project Description: (Continued)

Significance to Cancer Research: The advance in recovery of bone marrow cellularity following endotoxin treatment is of basic importance in cancer research. Induction of recovery in the irradiated marrow has hitherto been shown only following the injection of living cells, as nearly as one can judge from the literature. The importance of this observation is not limited to possible applicability of treatment, but extends to the general area of cell differentiation.

Proposed Course of Project: Efforts are being made to discover what processes are involved in the initiation of early recovery in the hemopoietic system of the irradiated animals. Sources of chemical fractions of the lipopolysaccharide are becoming available and an effort to define the fraction responsible for the initiation of hemopoietic recovery will be made. We shall attempt to make both a biological and chemical definition.

Part B included Yes [X] No [ ]

Serial No. NCI-659

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B.: Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, W. W., Marston, R. I. and Cornfield, J., Patterns of Hemopoietic Recovery in Irradiated Mice. Accepted for publication in Blood.

Invited to present some of these findings at the VII International Congress for Microbiology held in Stockholm, Sweden in August of 1958.

G. Brecher, W. W. Smith and E. P. Cronkite, Strahlenschutz durch Granulozyten, Chapter in "Physiologie und Physiopathologie der Weissen Blutzellen" Edited by H. Braunsteiner, Med. Univ. Klinik in Wien. Georg Thieme Verlag, Stuttgart.

Honors and Awards relating to this project:

None

Serial No. NCI-671

1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Protective Effect of a Colchicine and Related Compounds in Animals Exposed to X-Radiation

Principal Investigator:

Willie M. Smith, Ph.D.

Other Investigators:

Ilo N. Alderman and Ruth G. Simpson

Cooperating Units:

None

Man Years (calendar year 1959):
Total: 4/5
Professional: 1/5
Other: 3/5

Project Description:

Objectives: To determine the effects of colchicine and some of its derivatives on survival and hemopoiesis in irradiated animals.

Methods Employed: The material to be tested is injected at the desired times in relation to irradiation and studies are made on bone marrow, circulating cells and survival with particular concern for any evidence of alteration of marrow sensitivity to radiation.

Major Findings: Mortality is significantly reduced when these compounds are administered before (but not after) irradiation. Major differences in response are related to age, species and time of administration. Bone marrow recovers its cellularity and peripheral lymphocytes, granulocytes, platelets and hemoglobin concentration recover earlier under the conditions which produce an increase in survival. The observation that very young animals do not respond favorably to this treatment may be a clue to the mechanism involved.

Significance to Cancer Research: Any findings which are concerned with sensitivity to radiation and recovery from its damaging effects are of interest in cancer research and this project qualifies under both categories.

Project Description: (Continued)

Proposed Course of Project: This and related problems dealing with recovery of hemopoiesis will be studied with the view of elucidating mechanisms of action. Techniques will include the uptake of tritiated thymidine and special cytological preparations. Much of this material is ready to be written up for publication.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B.: Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, W. W., Protective Effect of a Colchicine Derivative in Mice Exposed to X-radiation, Science, 127, 340-341, 1958.

Smith, W. W. and Cornfield, J., Extending the Range of Dose-Effect Curves for Irradiated Mice, Science, 128, 473-474, 1958.

Smith, W. W., Induced Recovery of Blood-Forming Tissue in Irradiated Animals. Presented at the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, Switzerland, 1958.

Honors and Awards relating to this project:

None

Serial No. NCI-674
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Synthesis of Antibody in C_3H mice bearing the Transplantable Plasma Cell Tumor, X-5563.

Principal Investigator:

Falconer Smith

Other Investigators:

Marie M. Grenan, John Owens

Cooperating Units:

None

Man Years (calendar year 1959)

Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: Characterization of the immune mechanism in mice bearing the transplanted plasma cell tumor, and to determine combined effects of radiation and specific antibody on tumor growth and development.

Methods Employed: 1) Serums of mice immunized both with particulate (sheep erythrocytes) and soluble (bovine serum albumin) some time after tumor transplant are compared with their non-tumor bearing controls as to specific antibody content. 2) Serum titers are estimated by sensitive immunochemical tests involving either agglutination of tanned sensitized erythrocytes or their hemolytic power. 3) Passively transferred antibody is studied in tumor bearing mice to compare its half life with that in control mice.

Major Findings: 1) Capacity to form antibody both to particulate and soluble antigen can be correlated with size (weight) of the tumor. 2) This correlation does not extend to the secondary response, thus a well defined secondary immune response, may be elicited in mice bearing tumors of 5 grams weight or more. It [illegible] to detect antibody in mice bearing X-5563 tumors [illegible]

Major Findings: (Continued)

hemodilution since blood volumes with tumors over 5 grams average only about 1/3 more than their controls. 4) No evidence was found for possible serum components which would interfere with antibody determination. 5) Half life of passively transferred antibody is about the same in tumor bearing mice as in their controls, hence antibody, if formed, should be detectable. 6) 2000 r tumor dose in a single exposure caused regression of a tumor.

Significance to cancer research: Development of a significant specific antibody useful in treating the tumor as an adjunct of radiation therapy is predicted on a thorough understanding of the nature of protein synthesis in the presence of this tumor.

Proposed course of the project: Development of a specific antibody to the tumor will be attempted as an adjunct to radiation therapy.

Part B included Yes ☐ No ☒

Serial No. NCI-676
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

An Investigation of the Relationship between Cell Division and Irradiation Response to X-rays and Ultraviolet in Yeast

Principal Investigator:

Mortimer M. Elkind, Ph.D.

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Radiation injury, as evidenced by the loss of self reproductivity or the production of viable mutants, is conceptually associated with discrete sites. In yeast, conflicting reports exist with regard to the anatomical location of these sites. While most evidence favors the nucleus as the region of primary x-ray damage, RNA containing cytoplasmic particulates have been considered to be the primary UV sites. The latter view, in particular, had been strengthened by the apparent absence of a UV insensitive "tail" on previously reported UV survival curves. The x-ray survival curves of haploid through tetraploid yeasts have a radioinsensitive tail which has been shown to be due to the dividing moiety in the population.

The object of this research was to undertake a study of the UV response of a mixed population of yeast cells (dividing and non-dividing) in order to examine in detail the question of the presence or absence of a UV insensitive moiety.

Project Description: (Continued)

Methods Employed: The methods employed consisted of the production and detailed analysis of UV and x-ray survival curves, and the effects on the preceding of various permutations of x-rays, UV, and visible light (photoreactivation). The cell lines used were a pair of respiring strains (haploid and diploid) plus a non-respiring haploid subline. Both haploid lines can be lethally effected by exposure to visible light. See report No. NCI-636.

Major Findings: a) In contrast to UV survival curves published by others, we found that carefully performed experiments revealed that the UV curve, as well as the X-ray curve, is compound in nature. Although in the case of UV, the inflection in the survival curve is hardly as pronounced as in the case of X-rays nevertheless we showed that the inflection is real and results from the fact that cells in division are less sensitive to UV as well as to X-rays. b) Through the derivation of a mathematical theorem, we showed that the reduced UV sensitivity of dividing cells must result from a basic change(s) in the UV sensitive site structure of the cell. This agrees with conclusions reached by others in connection with the site structure sensitive to X-rays. c) The existence of differential sensitivities to UV exposure in a mixed population was also demonstrated in connection with mutation production and photoreactivation of UV lethality. And d) while quantitative differences were in evidence, the results obtained were general and independent of the ploidy, respiration competence, and visible light sensitivity of the cell lines used.

Significance to Cancer Research: A knowledge of the sites and mode of action of radiation at the cellular level is necessary for a detailed understanding of radiation effects. These latter effects, when understood, may expedite the use of radiation as a tool in biological research in general. More specifically, their understanding will play a role in explaining and controlling the carcinogenic and carcinocidal action of radiation.

Proposed Course of Project: It is proposed to extend these findings to an examination of the extent of overlap of sites sensitive to UV and X-rays through the use of additivity experiments.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Elkind, M. M. and Sutton, H., The Relationship between Division and X-Ray Sensitivity, Ultraviolet Sensitivity, and Photoreactivation in Yeast, Radiation Research, 10, 283-295, 1959.

Honors and Awards relating to this project:

None

Serial No. NCI-681
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Applications of Some Tissue Culture Techniques to Radiation Research

Principal Investigator:

Robert E. Bases, M. D.

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: Exploiting single cell techniques previously developed to detect materials capable of altering the radiation response of mammalian cells.

Methods Employed: Single cell techniques modified after Puck were applied. HeLa cells were used in the assays.

Major Findings: 1) Actinomycin D enhanced the killing effects of X rays on single cells exposed to the drug just before or after irradiation and during clone formation.

2) Cells irradiated in medium having 85% of the water replaced by D_2O were made more sensitive. Exposure to such medium alone sterilized normal cells upon progressive exposure. Survivors of D_2O exposure were abnormally sensitive to subsequent irradiation.

3) Materials tested which failed to modify the radiation response of mammalian cells are: catalase, ribonuclease, spermine.

4) The mechanism of action to actinomycin D is unknown. These findings have been incorporated in a paper submitted to Cancer Research.

Significance to Cancer Research: Actinomycin D appears to deserve critical evaluation as an adjunt to radiation therapy in human cancer therapy.

Proposed Course of Project: 1) It is suggested that actinomycin D be evaluated in animal or/and human cancer chemotherapy as an adjunct to radiation therapy.

2) The effect of actinomycin D and other materials on the post-irradiation status of cells deserves further study. This may lead to an understanding of repair mechanisms in the post irradiation status of cells.

Part B included Yes ☒ No ☐

Serial No. NCI-681

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Paces, R. E., Some Applications of Tissue Culture Methods to Radiation Research, Cancer Research, 19, 311-315, 1959.

Honors and Awards relating to this project:

None

Serial No. NUI-682
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Sites of Action of Lethal Irradiation in Dividing Yeast Cells

Principal Investigator:

Mortimer M. Elkind, Ph.D.

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959):
Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: Having found, as described in report No. NUI-676, that the general characteristics of both the UV and X-ray survival curves of yeast are consistent with the presence of overlap between the sites sensitive to both types of irradiation, an examination of the existence and degree of this possible overlap was undertaken. This examination was limited to the dividing cell moiety of a mixed population primarily because of the insensitive response of such cells, particularly to X-rays, is anomalous. This anomaly has been mainly responsible for the view that UV and X-ray sites are anatomically or at least functionally separated in dividing cells. Of course, are questions of first order in cellular radiobiology; namely where and what are the primary sites of lethal irradiation damage in living cells.

Methods Employed: The basic method involved consisted of the use of coupling or additivity experiments in which different combinations and permutations of UV, X-ray, and visible light exposures (for photoreactivation and photolethality) were employed. The underlying idea stemmed from the large number of instances in which it has been found that UV (254 mμ) effects which can be photoreversed are nuclear and have nucleic acid (NA) action spectra. Hence UV and X-ray effects that

Project Description: (Continued)

are coupled and which can be uncoupled by visible light in all likelihood involve nuclear NA as the sites common to both irradiations. To help insure that the conclusions reached were general, three cell lines were used. These were genetically related diploid and haploid lines, both respiring, and a non-respiring haploid subline of the preceding. Both haploid lines were shown to be able to be lethally effected by exposure to visible light. (See report No. NCI-636)

Major Findings: a) In addition to the mathematical proof described in report No. NCI-676, it was shown experimentally that the reduced UV sensitivity of dividing cells was not artifactual in nature. That is, it did not result, for instance, from a larger amount of specific but non-active absorption of the 254 mμ line due to the possible presence of more NA in dividing cells. b) It was shown that there is a large measure of overlap in sites sensitive to both UV and X-rays and that the coupling produced by pre-UV exposures could be undone concomitant the photoreversal of lethality produced by the UV. c) It was shown that it was quite unlikely that there exist two (or more) sets of sensitive sites in a dividing yeast cell; one set responsible for lethality, and the other responsible for coupling with X-ray sites. d) Photolethal effects, which were shown to be due to the development of a hemoprotein-like chromophore (report No. NCI-658), do not couple with UV or X-rays. Hence, in all likelihood these effects are cytoplasmic. e) While quantitative differences were observed, the conclusions which followed from these results were independent of the ploidy, respiration competence, and visible light sensitivity of the cell lines used. And f) the most consistent interpretation of these results is that in dividing yeast cells the sites sensitive to UV and X-rays are in appreciable measure nuclear and probably consist of chromosomal DNA.

Significance to Cancer Research: A knowledge of the sites and mode of action of radiation at the cellular level is necessary for a detailed understanding of radiation effects. These latter effects, when understood, may expedite the use of radiation as a tool in biological research in general. More specifically, their understanding will play a role in explaining and controlling the carcinogenic and carcinocidal action of radiation.

Proposed Course of Project: Depending on the availability of equipment and personnel, it would be desirable to perform action spectra on the specific coupling effects observed.

Part B included: Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Elkind, M. M. and Sutton, H., Sites of Action of Lethal Irradiation: Overlap in Sites for X-Ray, Ultraviolet, Photoreactivation, and Ultraviolet Protection and Reactivation in Dividing Yeast Cells, Radiation Research, 10, 296-312, 1959.

Honors and Awards relating to this project:

None

Serial No. NCI-685
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Thymidine Uptake in Hemopoietic Tissue Induced to Recover Following X-irradiation

Principal Investigators:

Willie W. Smith, Ph.D. and Victor Bond, Ph.D.

Other Investigators:

Ilo M. Alderman and Ruth G. Simpson

Cooperating Units:

Brookhaven National Laboratory

Man Years (calendar year 1959):
Total: 4/5
Professional: 1/5
Other: 3/5

Project Description:

Objectives: To discover processes concerned with post-irradiation recovery of blood-forming tissues.

Methods Employed: Autoradiography of hemopoietic tissue after treatment with thymidine to determine the presence of cells capable of mitosis.

Significance to Cancer Research: General significance in field of cell proliferation. Specific interest in connection with damage to blood-forming tissue following exposure to radiation.

Proposed Course of Project: Progress in this project has been slow due to pressure of other committments and technical difficulties - a specific course cannot be outlined until thorough studies of the preparations on hand have been made but we plan to proceed along the lines already indicated.

Part B included Yes ☐ No ☒

1. Radiation Branch
2. Rad. Biol. Lab.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Recovery of Ability to Form Antibodies in Irradiated Mice Pretreated with Endotoxin or Colchicine

Principal Investigators:

Willie W. Smith, Ph.D., Joanne Hollcroft and Jerome Cornfield

Other Investigators:

Ilo M. Alderman and Ruth G. Simpson

Cooperating Units:

School of Public Health and Hygiene, The Johns Hopkins University, Baltimore, Maryland

Man Years (calendar year 1959):
Total: 4/5
Professional: 1/5
Other: 3/5

Project Description:

Objectives: In study of recovery processes it is important to see how various hemopoietic functions are related. The question to be answered here is whether or not ability to react against foreign tissue is associated with the advance in recovery time of cellular elements as seen following isologous or heterologous bone marrow injection or treatment with colchicine or endotoxin.

Methods Employed: Transplantation of homologous ascites at intervals during recovery from irradiation in treated and untreated animals and observing response.

Major Findings: (a) Recovery of transplantation immunity to ascites cells of DBA origin in $(A \times F_1)$ mice following just sub-lethal irradiation was evident at a time which corresponded fairly closely to the time of recovery of other hemic elements: granulocytes, erythrocytes, lymphocytes and platelets. (b) An injection of isologous bone marrow did not hasten recovery but

Project Description: (Continued)

an injection of young spleen cells did hasten recovery of this immune mechanism. (c) A pre-irradiation injection of bacterial endotoxin did not significantly alter the time of recovery but an injection of colchicine caused a significant delay in recovery. (d) A significant gain in statistical information is achieved by utilizing survival times as well as survival percentages in analysis of the data. This is achieved by a procedure which is being worked out by Smith and Cornfield.

Significance to Cancer Research: Recovery processes after damaging radiation are of general interest because of the widespread use of radiation in cancer therapy. Recovery of this immune mechanism is of particular interest for if it can be delayed while other recovery processes advance, successful homologous transplantations might be more readily achieved than in the untreated irradiated case.

Proposed Course of Project: Additional experiments with the object of delaying recovery of transplantation immunity are contemplated.

Part B included Yes ☐ No ☒

Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Recovery Processes and Effects of Endotoxin and Colchicine Treatments on Recovery in Very Young Animals

Principal Investigator:

Willie W. Smith, Ph.D.

Other Investigators:

Ilo M. Alderman and Ruth G. Simpson

Cooperating Units:

None

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 4/5
Professional: 1/5
Other: 3/5

Project Description:

Objectives: To broaden the studies we are making of recovery from radiation damage.

Methods Employed: See related projects NCI-659, 671.

Major Findings: (a) We have recently found that much, if not all, of the difference in survivorship of weanling as compared to adult mice can be attributed to a later hemopoietic recovery in the young than in the older animals. (b) It appears that young mice (5 wk. old) are responsive to endotoxin but not to colchicine pretreatment.

Significance to Cancer Research: A better understanding of the factors influencing damage and recovery is of obvious significance in an area where radiation is used as extensively as in cancer therapy. These problems are also of general biological importance.

Proposed Course of Project: This involves primarily, comparative studies of hemopoietic recovery under different conditions as regards to (a) age, (b) species and (c) type of treatment.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

The Study of Basic Virus-Cell Interactions Using Quantitative Tissue Culture Techniques

Principal Investigator:

Royce Z. Lockart, Jr., Ph.D.

Other Investigators:

None

Cooperating Units:

Laboratory of Cell Biology,
NIAID

Man Years (calendar year 1959):
Total: ½
Professional: ½
Other: 0

Project Description:

Objectives: To carry out a quantitative study of the virus-cell relationships in the phenomenon of virus interference. The study is to gain insight on the effects of viruses on cells, the role of interference on the formation of virus carrier cultures, and to obtain the parameters of the interference system necessary to carry out studies on the intracellular nature of the interference.

Methods Employed: Methods employed have been those outlined for the quantitative assay of viruses (published: "Factors Influencing the Interaction of Western Equine Encephalomyelitis Virus and Selected Host Cells," J. Inf. Dis. 103: 165, 1958) and for the qualitative manner of handling tissue cultures while determining their ability to form clones.

Major Findings: Previous work showed that Western Equine Encephalomyelitis Virus inactivated at 37° can protect cells from the cytopathic effects of fully virulent homologous virus. Data obtained suggest that cells so protected are unable to produce virus until they undergo several generations of growth after which they become once again fully susceptible. About 50 per cent of cultures consisting of a protected population of cells became virus-carrier cultures when active virus was

Project Description (Continued)

added prior to their reversion to the susceptible state. Carrier cultures consisted of about one per cent infected cells in the presence of protected cells. The supernatant fluid from a carrier culture contained an excess of protective material. These results are consistent with the hypothesis that the interference phenomenon is responsible for the induction of the virus-carrier state and the prolonged maintenance is the result of a dynamic equilibrium between interference and virus production.

Significance to Cancer Research: The investigation is designed to attempt to understand the nature of cell changes brought about by inactive viruses which are capable of protecting cells against the cytopathic actions of other viruses. An understanding of the metabolic alterations effected in cells by an infective virus and a manner in which these can be changed to protect the cells will allow a rational approach for the development of substances effective in combatting virus infections, perhaps tumor viruses as well as cytopathic viruses.

Proposed Course of Project: To carry out experiments investigating the biochemical changes caused by infection and interference to attempt to find if interference is due intracellular biochemical inhibition of virus multiplication.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part E: Honors, Awards, and Publications

Publications other than abstracts from this project:

R. Z. Lockart, Jr.,
The Production and Maintenance of a Virus Carrier State: The Possible Role of Homologous Interference, Virology, in press.

Honors and Awards relating to this project:

Guest lecture for the Tissue Culture Course sponsored by the Long Island Biological Association at Cold Spring Harbor, New York

Accession No. 1011-59
1. Radiation Effects
2. Rad. Biol. Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Repair of X-ray Damage in Cell Lines of the Chinese Hamster Grown in Culture

Principal Investigator:

Mortimer M. Elkind, Ph. D.

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959)
Total: 2
Professional: 1/3
Other: 1-2/3

Project Description:

Objectives: To determine whether or not sublethal damage accumulated by mammalian cells surviving X-irradiation is heritable.

Methods Employed: Improved single cell tissue culture techniques were used for purposes of: 1) making dilute cell suspensions; 2) irradiating under controlled conditions; and 3) assaying for surviving cells. Soft-five Kv X-rays were used.

Major Findings: 1) Sublethal damage is not transmitted to the progeny of surviving cells. 2) After an acute exposure to X-rays, surviving cells rapidly repair their damage before their first division post-irradiation. 3) The kinetics of recovery displays at least one maximum followed by a minimum. 4) Repair of damage is expressed by the reactivation of sensitive sites inactivated by the X-ray exposure. 5) Repair of damage does not involve a time delay. 6) The system responsible for recovery in surviving cells is not saturated by repeated exposures. That is, surviving cells can repair

Project Description: (Continued)

repeated cycles of damage and repair. 7) The magnitude of recovery as a function of time is dependent upon the recovery medium. 8) In spite of the disproportionate synthetic capacities of lag phase cells compared to log phase cells, recovery in both types of cells is essentially the same.

Significance to Cancer Research: The repair of sublethal X-ray damage is a new finding of considerable importance with regard to the nature and location of the sites in a cell sensitive to lethal irradiation. The application of this finding to biomedical and particularly cancer research are many and include questions relating to: 1) radiation therapy; 2) mutagenesis; 3) dose rate dependence of radiation effects; 4) general mammalian radiobiology; 5) the cytogenetics of radiation damage and repair; and 6) the biochemistry of radiation damage and repair.

Proposed Course of the Project: The documentation of the major phenomenon has been completed. A number of ancillary projects aimed at a more general understanding of lethal and recovery effects in mammalian cells are planned or in progress. (See Project Numbers NCI-69I(a),69I(b),69I(c),[illegible]

In addition, it is of particular interest to pursue the aspects of these findings that relate to radiation therapy. This should involve work on a cellular and an animal level.

Part B included Yes ☒ No ☐

Individual Project Report
Calendar Year 1959

Part B.: Honors, Awards, and Publications

Publications other than abstracts from this project:

Elkind, M. M. and Sutton, H., X-Ray Damage and Recovery in Mammalian Cells in Culture, Nature, 184, 1293-1295 Oct. 24, 1959

Honors and Awards Relating to this Project:

Invited Lectures:

Title: "X-Ray Damage and Repair in Mammalian Cells Grown in Culture"
Place: Biology Division, Oak Ridge National Lab.; July 16, 1959

Title: "X-Ray Experiments with Mammalian Cells"
Place: Naval Radiological Defense Lab; San Francisco, Calif; Sept. 3, 1959

Title: "Cellular Recovery Related to Radiation Therapy"
Place: Radiology Dept., University of California Medical Center, San Francisco, Calif.; Sept. 3, 1959

Title: "Cellular Aspects of Radiation Therapy"
Place: 45th Annual Meeting of the Radiological Society of North America; Chicago, Ill., Nov. 17, 1959

Title: "Radiation Genetics"
Place: National Research Council Committee on Radiobiology, invited conference on radio- and molecular biology, Gould House, Ardsley, N. Y., Dec. 3, 1959.

1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

The Dependence of Lethality on X-ray Dose Rate in Chinese Hamster Cells Grown in Culture

Principal Investigator:

Mortimer M. Elkind, Ph. D.

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: The observation that surviving cells to X-irradiation rapidly repair their damage without a time l.c (See Project No. NCI-691) suggests that lethality should be dose rate dependent. Specifically, this observation suggests that at reduced dose rates the killing effect (suppression of unlimited division) should be reduced.

Methods Employed: Improved single cell tissue culture techniques were used for purposes of: 1) making dense cell suspensions; 2) irradiating under controlled conditions; and 3) assaying for surviving cells. Fifty-five Kv X-rays were used.

Major Findings: This work is in progress. Thus far we have found that reduce dose rates have a greater rather than lesser killing effect on Chinese hamster cells grown in culture as indicated by the mean lethal dose. The dose rate range from 720 rad/min to 9 rad/min is under investigation. Preliminary data indicate that the latter dose rate increases the mean lethal dose by about a factor of 2 compared to the former. However, although the mean lethal dose decreases with decreasing dose rate, the hitness number increases.

Project Description: (Continued)

Significance to Cancer Research: The anomolous nature of these findings are of significance primarily as they relate to the information derived from Project #NCI-691.

Proposed Course of Project: This work will be pursued in order to seek an explanation of these seemingly anomolous results. Due to practical limitations (equipment, personnel, and to some extent techniques) the range of dose rates which can be examined with good experimental control is limited. The importance of understanding these results justifies considerable additional effort.

Part B included Yes ☐ No ☒

2. Rad. Biol. Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Division Delay, Growth Rate, and Radiosensitivity of Chinese Hamster Cells Which Survive X-Irradiation.

Principal Investigator:

Mortimer M. Elkind, Ph.D.

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959):

Total: 2/3
Professional: 0
Other: 2/3

Project Description:

Objectives: Sublethally irradiated cells repair their damage rapidly (see NCI-6?1). The purpose of this study is to determine whether or not: 1) recovery is associated with division in surviving cells; 2) post irradiation growth rate of surviving cells is dose dependent; and 3) radiation sensitivity of surviving cells is dose dependent.

Methods Employed: In addition to those listed for Project [illegible] the following specific methods were employed: 1) in vitro observation of the onset of division in surviving cells using phase microscopy; 2) determination of the termination of delay and subsequent growth rate using a colony spreading technique (resuspension); and 3) measurement of delay, growth rate, and radiation sensitivity post-irradiation using a novel x-ray technique and the usual visible colony formation.

Major Findings: 1) Lethally irradiated cells grow as rapidly as controls for at least a few divisions depending on the dose. In contrast to this, surviving cells suffer a delay which increases with the dose they received. 2) After post-irradiation delay, surviving cells grow as rapidly as the controls. And 3) the radiation sensitivity of survivors after the onset of division is constant and about the same as that of lag phase cells.

Significance to Cancer Research: Mitotic index in suspension radiobiology has often been used as an index of survival. To the extent that these observations are applicable to in vivo situations, mitotic index as a measure of viability can be very misleading. Similarly, the observations that growth rate and the radiation sensitivity of surviving cells are essentially independent of the x-ray dose to which they were exposed is of fundamental interest in the basic and applied areas of radiobiology.

Proposed Course of Project: The quantitation of these observations as a function of dose is best performed with log phase cells. In addition, the division delay dependence on dose is required for (NCI-691 (d)). For these reasons and others, the technology of growing Chinese hamster cells in suspension (log phase) warrants development and will be vigorously pursued.

Part B included Yes ☐ No ☒

Individual Project Report
Calendar Year 1959

Part A.

Project Title:

The [illegible]ment of the Dependance of X-Ray Lethality on [illegible] Damage in Chinese Hamster Cells Grown in Culture.

Principal Investigators:

Mortimer M. Elkind, Ph. D.

Other Investigators:

Charles Greenblatt, M. D.

Cooperating Units:

Photobiology Section, Physical Biology Laboratory, [illegible]

Man Years:

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: As a result of a number of radiation [illegible] cultured mammalian cells, Puck has concluded that [illegible] results from the production of observable chromosomal [illegible] This conclusion, if correct, is of basic importance and has many implications. The objective of this study is to see to what [illegible] Puck's conclusion is justified in Chinese hamster cells [illegible] to normal karyotype

Methods Employed: Quantitative cytogenetic observations of control and irradiated cells are made in the [illegible] lethality and recovery effects described in Project Nos. NCI-691, NCI-691 (a), NCI-691 (b).

Major Findings: This work is in progress. One of the [illegible] reasons for starting this study was the recovery observed [illegible] described in NCI-691. The rational is the following: 1) surviving cells immediately after exposure are damaged cells. If the accumulation of chromosomal damage kills a cell, [illegible] cells immediately after exposure should possess multiple chromosomal damage. 2) Surviving cells rapidly repair their sublethal damage before division (see NCI 691 and NCI [illegible]). Therefore unless irradiation induces a state of lability with

Major Findings: (Continued)
regard to the display of chromosomal damage; surviving cells after recovery and particularly their progeny cannot show any chromosomal damage. 3) Surviving cells suffer a division delay during which (in interphase) they repair their damage. In contrast to this, lethally irradiated cells divide as rapidly as the controls for at least a few divisions (see NCI-G71(b)). Hence, cytogenetic observations of all cells irradiated might display various degrees of damage depending upon whether surviving or non-surviving cells happen to be observed which, in turn, depends on whether or not recovery and post-irradiation delay have terminated. However, as these observations are made before division delay is over, which is experienced only by surviving cells, then all aberrations observed will be in lethally irradiated cells. If unlimited division in these cells is suppressed because of observable cytogenetic damage, then all mitoses should display chromosomal abberations and indeed the frequency of these should be directly correlatable with survival as a function of dose.

Thus far our studies, conducted with lag phase cells, have shown that only a fraction of the mitoses observed during division delay have any observable chromosomal aberrations. Others have 1, 2, etc. aberrations but with frequencies too low to explain shape of the survival curve in detail.

Significance to Cancer Research: If further work bears out our initial observations that lethally irradiated cells do not display on a quantitative basis, chromosomal damage correlatable with lethality, then the chromosomal hypothesis of [illegible] is in doubt. This would not mean that x-rays cannot produce aberrations or that many such aberrations do not constitute [illegible] urations. Rather, this would mean that observable chromosomal damage can be at best only one mechanism responsible for cell death. Clearly, subtle, optically undetectable chromosomal damage or in the limit, genic damage cannot be ruled out on the basis of cytogenetic observations. By the same token, however, non-genic damage related to viability becomes a significant possibility by implication.

Proposed Course of Project: The quantitation of [illegible] tion in mitosis would be best performed with single lag phase cells for technical reasons. However, the techniques required for the production of such cells require development before the cytogenetic aspects are pursued further.

Part B. included Yes ☐ No ☐

Serial No. NCI-691-C
1. Radiation Branch
2. Rad. Biol. Sect.
3. Bethesda, Maryland

DHEW-NIH
Individual Project Report
Calendar Year 1959

Part I

Project Title:

The Dependence or Independence of Recovery in Cultured Mammalian Cells Surviving X-Irradiation and Their Radiosensitivity as Mitigated by Drug Action

Principal Investigator:

Mortimer M. Elkind, Ph. D.

Other Investigators:

William Mohler, M. D.

Cooperating Units:

Clinical Pharmacology and Experimental Therapeutics Section, General Medicine Branch, NCI

Man Years (calendar year 1959)
Total: 1/3
Professional: 0 (M.M.E.)
Other: 1/3

Project Description:

Objectives: In Project No. NCI-691, recovery in cells surviving X-irradiation was described, and it was further reported that recovery was found to be independent of the physiological state of the cells. In general, radiosensitivity is known to be modifiable via the action of drugs. For instance, aminoethylisothiuronium bromide (AET) is known to decrease radiosensitivity by about 2x. This study is one aimed at examining the degree to which recovery is general in mammalian cells. Specifically the question posed is: Do surviving cells recovery qualitatively and quantitatively to the same extent and irrespective of their probability of survival for a given dose? In the same cell line, therefore, we will inquire whether or not the mitigating action of drugs on the probability of survival is reflected in the repair of sublethal damage in surviving cells. Since the dependence of radiosensitivity on drug action is in itself of considerable interest, a second objective in this work is to examine the influence on survival of different

Project Description: (Continued)

Methods Employed: Improved single cell tissue culture techniques were used for purposes of: 1) making dense cell suspensions; 2) irradiating under controlled conditions; and 3) assaying for surviving cells. Fifty-five Kv X-rays were used.

Major Findings: This work is in progress. Toxicity studies have been performed with AET, 5-bromouracil, and 5-fluoro-deoxyuridine. Thus far, only AET has been examined and only with regard to its influence on radiosensitivity. As found in other systems, AET decreases the radiosensitivity by about 2x.

Significance to Cancer Research: The mitigating action of drugs is of importance with regard to the identification of the sites of action of lethal irradiation and the radiation response of malignant tissue. Equally important from a basic and applied point of view is a knowledge of the possible influence of the action of drugs on recovery. For instance, if a given drug mitigates (perhaps even decreases) the radiosensitivity of malignant cells it might still have important uses in therapy if it also represses recovery.

Proposed Course of Project: It is planned to examine the influences of several drugs which have been implicated as radiation mitigating agents.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

A Study of the Response of Human Cells (HeLa) to X-Rays

Principle Investigator:

Royce Z. Lockart, Jr., Ph. D.

Other Investigators:

Mortimer M. Elkind, Ph. D.

Cooperating Units:

None

Man Years (calendar year 1959):
Total: ½
Professional: ½
Other: 0

Project Description:

Objectives: Dr. M. M. Elkind using Chinese hamster cells has made detailed analyses of survival curves resulting from single and fractionated doses of X-rays. He has established that these cells have a threshold amounting to a hitness number between 0 and 2 and that survivors were able to repair their sub-lethal damage. The amount of damage suffered or the repair which occurs is related to the hitness number. Dr. Robert Bases, during preliminary studies on HeLa cells noticed that one of his cell lines changed from a hitness number of 2 to about 12. The purpose of this investigation was to make a comparative study of two lines of HeLa cells from the same parental stock with hitness numbers of two and five. Experiments were done to see if recovery from radiation damage was a general phenomenon rather than unique to Chinese hamster cells and to see what relationship exists between hitness number and recovery.

Methods Employed: The methods used were those for measuring survival by means of counting clones of cells resulting from the growth of single cells and for irradiating cells [illegible] to glass petri dishes. These methods are both amply described by Dr. M. M. Elkind.

Project Description: (Continued)

Major Findings: One Hela strain had a hitness number which varied from 3.3 to 0 with a mean lethal dose varying between 115 r. and 95 r. respectively. In response to a first dose of X-rays corresponding to about 10 percent survival, cells were capable of undergoing an amount of recovery about equal to their hitness number. This means the cells have regained their threshold and have the same sensitivity. The other strain of Hela had a hitness number of two for several months with a mean lethal dose of about 130 r, yet showed about 5 fold recovery. The reason for this has not yet been determined as the cell strain was not stable with regard to its radiation response and gradually shifted so that it was indistinguishable from the other strain.

These studies have confirmed Dr. Elkind's studies with the Chinese hamster cells, and have shown that the recovery phenomenon appears to be a general one. Indications are that both hitness number and sensitivity to X-rays are somewhat variable from experiment to experiment and at least in one cell line unstable.

Significance to Cancer Research: These studies are part of a program to determine the sites and kinds of damage which cells suffer as a result of X-ray insult. The understanding of the numerous parameters controlling the reactions of cells to ionising radiation is most important both from the fundamental understanding of its actions from a biological point of view and from the practical applications in treatment of tumors.

Proposed Course of Project: To attempt to understand why one cell strain became altered in its response to X-rays.

Part B Included Yes ☐ No ☒

[redacted]

Individual Project Report
Calendar Year 1959

[redacted]

Project Title:

Studies of effects of an antibody in relation to development of an ascites (P-388) plasma cell tumor.

Principal Investigator:

Falconer Smith

Other Investigators:

John Owens

Cooperating Units:

None

Man Years

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives: To expand preliminary observations on tumoricidal effects of antibody prepared against an ascites tumor.

Methods employed: Antibody prepared in rabbits against [redacted] cells freshly taken from the ascites fluid of the tumor [redacted] 7 days after transplant is intraperitoneally or intravenously injected at intervals of time after the tumor is inoculated.

Major findings: Preliminary observations indicate [redacted] of the antibody on the growth of the neoplasm, viz., [redacted] weights in groups of mice receiving 0.2 ml of rabbit antiserum intraperitoneally every other day from the day of [redacted]

[illegible] (Continued)

follows a less striking increase and survival time is prolonged by about 2 days as compared to saline treated controls inoculated with this rapidly lethal tumor.

Significance to cancer research: A specific antitumor preparation is possible outcome of this study and it is expected that what is learned from the properties of the preparation under study may lead to some understanding of naturally produced cytolytic agents.

Part B Included Yes ☐ No ☒

Serial No. NCI-62h
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Radiation Injury and Repair

Principal Investigator:

M. L. Andrews

Other Investigators:

Major James Shively

Cooperating Units:

Walter Reed Army Medical Center

Man Years:

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To gain insight into mechanisms by which ionizing radiations affect living tissues.

Methods Employed: Repair processes at high x-ray doses were studied by dose fractionation. A second phase involves studies on skin, animals of interest because their size approximate that of man.

Major Findings: Repair processes appear to be operative even at dose of 50,000 r.

Part B included Yes ☐ No ☒

Serial No. NCI-628
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Dosimetry of High Energy Radiation

Principal Investigator:

H. L. Andrews

Other Investigators:

Louis Cameron

Cooperating Units:

None

Man Years:

None

Project Description:

Objectives: To develop methods and instruments for measuring high intensity pulsed x-ray beam.

Methods Employed: No progress was made during 1959 due to lack of personnel.

Part B included Yes ☐ No ☒

Serial No. NCI-629

1. Radiation Branch
2. Rad. Physics Sect.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Project Title:

The Effect of Ionising Radiation on Amino Acids

Principal Investigator:

Charles R. Maxwell, Ph.D.

Other Investigators:

None

Cooperating Units:

None

Man Years: (calendar year 1959)
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: The objective of this project is to determine the mechanism of the chemical reactions induced by ionising radiation in aqueous solutions of amino acids. This is part of a long range program to accumulate information on simple systems of biological interest so that general principles may be ascertained and applied to complex systems which are not amenable to thorough, direct investigation.

Methods Employed: Solutions of amino acids are irradiated with ionising radiation and then analysed for the products formed. Since the products are sensitive to radiation it is necessary to determine the yield of each product as a function of dose to as low a dose as possible and to extrapolate these values to obtain the initial yield of the product at very low doses before secondary reactions distort the picture.

Once the products of the simple water-amino acid-radiation system are found the effect of the introduction of other chemicals into the system are studied. Substances are selected, in accordance with the rapidly expanding concepts of radiation chemistry, which are thought to accentuate or repress one or more of the various intermediate processes by which radiation is believed to produce its effects.

Project Description: (Continued)

Since the final chemical (and ultimate biological) effect is the result of the formation of a complex microscopically-heterogeneous mixture of excited molecules, H_2O_2, H and OH free radicals in discrete "tracks", the influence of the physical distribution of these active intermediates as well as possible differences in relative abundance and/or chemical nature is investigated by using different sources of radiation. To date 50 KV x-rays, 150 KV electrons, and 5 Mev alpha particles have been used.

One of the more important, if not the most important, active intermediates is the OH free radical. The role of this radical in the absence of the H free radical and the host of excited molecules in the ionization track is studied by chemically producing this radical in the system being studied. To date this has been accomplished by the action of Fe^{++} and H_2O_2.

Major Findings: The development of a gas chromatographic technique for the isolation, concentration and determination of micromolar amounts of acetic acid produced from glycine by small doses of irradiation has made possible some detailed observations on the mechanism for the production of this compound. Initial yields of acetic acid as a function of glycine concentration have shown that it, like NH_3 and HCHO but unlike HCOOOH, does not become independent of concentration as does the yield of products from most organic solutes.

Of more significance have been the mass spectrographic examination of the acetic acid produced when glycine was irradiated in D_2O. According to the mechanism proposed by Weeks and Garrison

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Boyd M. Weeks and Warren M. Garrison, Rad. Research 9, 291 (1958).

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acetic acid is formed by the reactions:

$$1)\quad H + NH_2CH_2COOH \Longrightarrow NH_3 + {}^{\circ}CH_2COOH$$

$$2)\quad {}^{\circ}CH_2COOH + NH_2CH_2COOH \longrightarrow CH_3COOH + NH_2\dot{C}HCOOH$$

In D_2O the corresponding reactions would be:

$$1)\quad D + ND_2CH_2COOD \Longrightarrow ND_3 + {}^{\circ}CH_2COOD$$

$$2)\quad {}^{\circ}CH_2COOD + ND_2CH_2COOD \longrightarrow CH_3COOD + ND_2\dot{C}HCOOD$$

and the acetic acid formed (after the removal of the exchangeable D on the carboxy group should be CH_3COOH. Mass spectrographic analysis of the acid isolated however has shown a mixture of 1/3 CH_3DCOOH and 2/3 CH_3COOH. Thus there must, at the least, be a second method for the formation of acetic acid.

Project Description: (Continued)

With ion exchange column chromatography, C^{14} labeled glycine, and liquid scintillation counting we have qualitatively confirmed the work of Weeks and Garrison as to substantial yields of aspartic and diaminosuccinic acids in air free irradiations. We observe one but not the second major unknown product reported by them. Since we have observed the yields of these products to be a function of dose, they will have to be investigated at low doses before their part in the entire mechanism can be evaluated. And of course the unknown product will need to be identified.

These results show that the mechanism proposed by Weeks and Garrison which seemed to account so beautifully for most of the data available to them at that time is, at best, only partly correct and that considerable more work is needed.

A major effort this year has been the design and procurement of a cobalt 60 irradiation facility which will be put in operation early next year.

Significance to Cancer Research: Although not directly connected with Cancer research, it is of great interest because it seeks an understanding of the mechanism for the effects of radiation which is used empirically as a tool in the clinical treatment and laboratory study of cancer.

Proposed Course of Project: Work will continue in an effort to fully elucidate the mechanism of the effect of radiation on glycine.

Specifically the work will be directed toward:

a) Identifying and determining the unknown product indicated by an incomplete material balance.

b) An investigation of the O_2 present system as well as the O_2 free system upon which our efforts in the past has been primarily concentrated.

c) An investigation of the effect of pH upon both the O_2 free and O_2 present system.

d) Study the role of disproportionation reactions by irradiating a pure d or l amino acid and assaying enzymatically for the optical isomer.

Part B included Yes [X] No [ ]

NRS-NIH
Individual Project Report
Calendar Year 1959

Part B.: Honors, Awards and Publications

Publications other than abstracts from this project:

Maxwell, Charles R. and Peterson, D. C., The Effect of α-radiation on Aqueous Glycine, J. phys. chem. 63, 935 (1959).

Honors and awards relating to project:

Dec. 29, 1958 - AAAS Meeting, Washington, D. C., presented invited paper at symposia on "Radiation Chemistry"

Dec. 29, 1959 - AAAS Meeting, Chicago, Illinois, presented invited paper at symposia on "Radiation Chemistry of Proteins and Amino Acids"

Serial No. NCI-637
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PAS-NIH
Individual Project Report
Calendar Year 1959

Part I.

Project Title:

Development of High-Intensity X-Ray Source

Principal Investigator:

Howard L. Andrews

Other Investigators:

Robert W. Swain

Cooperating Units:

None

Man Years:

None

Project Description:

Objectives: To obtain high intensity beams of x-rays, pulsed or steady from the 3 Mev generator.

Methods Employed: No progress was made in this problem during 1959 due to lack of personnel.

Part B included Yes ☐ No ☒

Serial No. NCI-680
1. Radiation Branch
2. Radiation Physics Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Radioactive Isotope Administration by Iontophoresse

Principal Investigator:

Howard L. Andrews

Other Investigators:

Louis Cameron and Dorothy C. Peterson

Cooperating Units:

None

Man Years:

Total: 1
Professional 2/3
Other: 1/3

Project Description:

Objectives and Methods Employed: A variety of isotopes were administered through the skin of guinea pigs by the application of electric current. Isotope distributions were determined by assaying skin sections and pertinent organs. The distribution of P^{32} seemed of sufficient interest to warrant an attempt at tumor treatment. Walker sarcomas in rats were treated by the local application of P^{32} by electric current.

Major Findings: Tumor concentrations of P^{32} several times higher than in normal tissue were readily obtained. Calculations indicated that doses of a few thousand rad were delivered to a good fraction of the tumor tissue. In some cases there was a temporary slowing of tumor growth and in one case a complete regression. In general, however, the results were unsatisfactory.

Significance to Cancer Research: There is probably little practical application to cancer treatment although other tumors might respond satisfactorily.

Proposed Course of Project: There seems little chance of improving the concentration ratio of the administered isotope. Although some more animal trials would be of interest they should have a low priority.

Part B included Yes ☐ No ☒

Project Title:

The Abstraction Reactions of Hydrogen Atoms in the Radiolysis of Aqueous Organic Solutions. Part I. Amines, Amino Acids, Amides, Peptides and Thiols.

Principal Investigator:

Dr. Peter Riess

Other Investigators:

Dr. Charles R. Maxwell
Mr. Bernard Burr

Cooperating Units:

Energy Metabolism Section, Laboratory of Physiology, National Cancer Institute

Man Years (calendar year 1959)

Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: The action of ionising radiation on aqueous solutions produces H atoms, OH radicals, hydrogen and hydrogen peroxide. In the absence of dissolved oxygen, H atoms may react with water-soluble organic solutes by abstracting H atoms from carbon, nitrogen, sulfur and oxygen to produce hydrogen and an organic free radical.

For solutes which have more than one site at which H atom abstraction may occur, there is at present insufficient information for predicting the site or sites of reaction. The objectives of this project are to study the mechanism of the abstraction reactions for such solutes and thus to determine the nature of the organic free radicals produced.

Methods Employed: When aqueous organic solutions are exposed to ionising radiation, hydrogen is produced by two distinct processes. The primary molecular hydrogen yield arises from the recombination of H atoms in a series of small regions of high radical concentration (known as "spurs") and is independent of added solute under certain conditions. Additional hydrogen is formed from the reactions of H atoms which diffuse out of the spurs and react with the organic solute.

Analogous processes occur when organic solutes dissolved in D_2O are irradiated and the primary molecular H_2 yield is known from previous studies.[1]

[1] F. S. Dainton: Rad. Res. Supplement I, 19, (1959)

Methods Employed: (Continued)

$g(D_2)$, the number of D_2 molecules formed per 100 ev absorbed, is equal to 0.39 for 280 kvp x-rays. Additional D_2 or HD is formed from the reactions of D atoms with the solute. In the present study solutes were deuterium labeled on nitrogen or sulfur by exchange in D_2O (99.5 atom %), leaving hydrogen stably bound to carbon under the conditions of the experiments. Thus for the case of acetamide dissolved in D_2O, hydrogen in excess of the primary molecular yield might be formed by reactions 1 or 2:

$$1)\quad D + CH_3CO\,ND_2 \xrightarrow{k_m} D_2 + \dot{C}H_2CO\,ND$$

$$2)\quad D + CD_3CO\,NH_2 \xrightarrow{k_c} HD + \dot{C}H_2CO\,ND_2$$

The hydrogen (D_2 + HD) produced during radiolysis of a degassed solution of acetamide (or of other solutes) was extracted on a Van Slyke apparatus and its isotopic composition determined by mass-spectrometry. From the observed yields of D_2 and HD [$G(D_2)$ and $G(HD)$], the relative contributions of reactions 1 and 2 can be measured. If both contribute appreciably, the ratio of the rate constants, k_m/k_c, can be calculated since

$$\frac{k_m}{k_c} = \frac{G(D_2) - g(D_2)}{G(HD)}$$

Major Findings: The observed yields, corrected for the primary molecular D_2 yield are summarized in Table I. The results show that D atoms react with glycine, acetamide and glycylglycine at neutral pD by abstracting from carbon and not from nitrogen. In strongly basic solutions, where the dipolar ion of glycine is converted to $ND_2CH_2COO^-$, an appreciable fraction of D atoms abstract from the $-ND_2$ group of glycine. A similar result is found for n-butylamine. The relative rate constants for abstraction from nitrogen vs. carbon are shown in Table I.

For substances containing the -SD group, n-butylthiol and cysteine, the formation of RS· radicals predominates in abstraction reactions. Thus for cysteine the rate of abstraction of deuterium from sulfur is approximately 14 times greater than the rate of abstraction of hydrogen from carbon.

An interesting aspect of the results of Table I is the fact that that fate of more than half of the D atoms produced by radiation in neutral solution is unaccounted for by abstraction reactions. In the case of glycine indirect evidence suggests strongly that H atoms can react with the dipolar ion to give ammonia and $\dot{C}H_2COO^-$ radicals.[2]

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[2] C. M. Maxwell, Annual Report, NCI-689, (1958, 1959)

A similar reaction appears to take place with amides. In the case of the [illegible], the missing D atoms probably react to produce D[illegible], which is known to be a major product in the radiolysis of [illegible].

Table I. — Relative Rate Constant for Abstraction Reactions at 25°C.

Solute	$G(D_2) - g(D_2)$	$G(HD)$	$\frac{k_x}{k_e}$ (a)	$\frac{k^*_I}{k^*_e}$ (b)
1. CH_3CONH_2	.03 ± .02	.64	<.08	<.12
2. $\overset{+}{N}D_3CH_2C\overset{-}{O}O$	.01 ± .02	.57	<.05	<.03
3. $\overset{+}{N}D_3CH_2CONHCH_2C\overset{-}{O}O$	.01 ± .02	.44	<.07	<.07
4. $ND_2CH_2C\overset{-}{O}O$	.12	.39	.31	.31
5. $CH_3(CH_2)_3ND_2$	.21	.90	.23	.69 (c)
6. $CH_3(CH_2)_3SD$	.45	.24	1.9	11.4 (c)
7. $\overset{+}{N}D_3CH(CH_2SD)C\overset{-}{O}O$	.99	.037	14.3	14.3 (d)

(a) ke refers to the rate of abstraction from carbon, kx to the rate of abstraction from elements other than carbon. From nitrogen for solutes 1 - 5; From sulfur for solutes 6 and 7.

(b) k^* is the rate constant per C-H or N-H bond.

(c) calculated assuming 6 equally reactive secondary H atoms.

(d) calculated assuming reaction of the tertiary H atom only.

Major Findings: Continued)

The results for glycylglycine are of significance for the aqueous radiation chemistry of proteins, since they indicate that the reactions of H atoms with the peptide bond will not produce free radicals with the odd electron on the nitrogen of the peptide bond.

Significance to cancer research: This project contributes to the knowledge of the initial free radical reactions which are a major part of the initial radiation damage in biological systems.

Proposed course of project: The rate of abstracting a deuterium atom from a -C-D or -N-D bond may be 5-10 times smaller than the rate of abstracting an H atom from a C-H or N-H bond. In order to evaluate the importance of this deuterium isotope effect, the radiolysis of some representative compounds (CD_3CONH_2 and $ND_3CH_2COO^-$) will be studied in light water.

The extension of the present approach to other functional groups (hydroxide and guanidinium) is being considered.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Service Radiation (Clinical Therapy)

Principal Investigator:

Kirkland Brace, M. D.

Other Investigators:

J. Robert Andrews, M. D.

Cooperating Units:

None

Man Years:
Professional: 2/3
Others: 1-1/2
Total: 2-1/3

Project Description:

Objectives: To provide radiation therapy for those patients requiring such treatment in the course of the disease for which they are being investigated by NIH research groups other than the Radiation Branch.

Patient Material: A total of 189 consultations were given to the various departments of this Institution during the past 12 months. A total of 184 courses of radiation therapy were administered.

Methods Employed: A wide range of photon energies were used and various methods of application were employed. Following are the number of courses of treatments at the various energies noted:

160 courses of 2 Mev irradiation
7 courses of 250 kilovolt radiation
6 courses of 140 kilovolt radiation
4 courses of 60 kilovolt radiation
5 courses of electron beam therapy
1 cervical radium application

Major Findings: Response to radiation therapy has been consistent with responses reported by others.

Proposed Course of Project: To continue.

Part B included Yes ☐ No ☒

Z01 CA [illegible]

1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH

Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Childhood Cancer

Principal Investigator:

Kirkland Brace, M. D.

Other Investigators:

J. R. Andrews, M. D. and Louis V. Avioli, M. D.

Cooperating Units:

None

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: 0

Patient Days (calendar year 1959):
approx. 200

Project Description:

Objective: The objective of this is to collect data on the treatment of malignant diseases of childhood.

Patient Material: All children under the age of 18 who have malignant tumors.

Major Findings: Malignant tumors of children are extremely rare and we have collected only 19 cases. We can only say at this point that histological diagnosis is particularly difficult and a very poor guide to the rapidity with which tumors will spread, and their radiation sensitivity. This program is significant to biological research only in that we hope to gather enough data from these extremely rare cases to classify these tumors and determine what place radiation therapy has in their treatment.

Proposed Course of Project: We propose to carry on with this project until we have gathered sufficient case material on which we can draw some type of conclusion.

Part B included Yes ☐ No ☒

1. Radiation Treatm.
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Time as a Modifier of Clinical Radiation Response

Principal Investigator:

J. Robert Andrews, M. D.

Other Investigators:

Herman D. Suit, M. D., Stanley E. Snaider, M. D., Kirkland C. Brace, M. D., Louis V. Avioli, M. D. and Gordon Sharp, M. D.

Cooperating Units:

None

Man Years (calendar year 1959):
Total:
Professional:
Other:

Project Description:

Objectives and Methods Employed: This project is an extension of NCI-2-654(c). The theoretical dosimetry, and general clinical aspects of this project were reported in detail in 1958 and no variations have been introduced or observed in 1959.

Patient Material and Major Findings: The patients included in this study now number 45 of which 40 had their treatment commenced 12 or more months ago. The median survival time of this group of 40 is 10 months. The patients included in the study are, in general, those with rather far advanced cancer of the head and neck. It is not likely that the median survival time of 10 months is worse than would have been attained had these patients been treated in a more conventional manner with a smaller dose given over a shorter period of time. Local cutaneous and other normal tissue reactions have been mild. In respect to the skin response the mild reactions are probably largely related to absorption characteristics of a 2 Mev X-ray beam rather than to the extended time of irradiation. In respect to other normal tissue reactions the opposite may be the case. This case material is presently being critically reviewed and worked up for publication.

Proposed Course of Project: Patient admissions to the project have been suspended until such time as the case material has been critically reviewed and worked up so that the project can be adequately reconsidered.

Part B included Yes [X] No [ ]

Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Andrews, J. E. and Snelder, S. E., The Modification of the Radiation Response, Am. J. of Roentgenol. and Radium Therapy, 81: 485, 1959.

Honors and Awards relating to this project:

None

Shak No. [illegible]
1. Radiation
2. Rad. Ther. Serv.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Distribution of S-35 in Human Tissues and Tumors and the Clinical Effects of Large Doses of S-35.

Principal Investigator:

J. Robert Andrews, M. D.

Other Investigators:

Lawrence Schlacter, M. D., James A. Rose, M. D., Jack Levin, M. D., Richard Swarm, M. D., and Hazel Gump, A. B.

Cooperating Units:

NCI-Pathologic Anatomy Branch, NCI-523

Man Years (calendar year 1959): Patient Days (calendar year 19[illegible]

Total:
Professional:
Other:

Project Description:

Objectives and Methods Employed: The conceptual structure of the project remain the same as reported in detail as NCI-[illegible] for calendar year 1958. The study has been severely handicapped because appropriate patients have not become available.

Patient Material: One other case has been studied. This is the case of an elderly male presenting with massive thoracic wall, intrathoracic, and extrathoracic chondrosarcoma. Studies of the S-35 uptake in tumor and skin showed concentration of the S-35 in the tumor to be greater than that in the skin by factors from 5 to 30 depending upon the time or, perhaps, only the normal variation, of the biopsies. A preliminary distribution study was repeated following the administration of growth hormone and neither the relative nor the absolute concentration of S-35 in tumor or skin changed. Following the preliminary studies 388 mc. of S-35 were given and two months later another dose of 335 mc. was given.

Patient Material: (Continued)

was only slight depression of the white and platelet counts in the period between the two doses but 4 weeks following the second dose there was marked depression of the platelets and moderate depression of the peripheral white cells. These recovered after two months but 6 months after the second dose the patient was found to have a severe anemia and thrombocytopenia with petechiae. This is presumably related to persistence of hemopoietic depression by the S-35 but clinical studies for the absolute identification of the cause of the peripheral blood findings have not been completed. On the other hand, a patient treated similarly in the previous calendar year remains well insofar as normal hemopoietic function is concerned and without symptoms insofar as the local mass of tumor of the pelvis is concerned. Both patients received local, intensive external beam x-ray therapy to the lesions following the sulfur studies. In the case of the male with the thoracic wall neoplasm this treatment became necessary because of recurrence of uncontrolled, ulcerative tumor of the external chest wall.

Proposed Course of Project: These studies will be pursued very much as has been outlined above and in previous reports if some material becomes available. It has become apparent that careful attention has to be given to the matter of hemopoietic reserve and we are prepared to do the studies necessary to an understanding of the effects on this reserve.

Part B included Yes ☒ No ☐

Part E: Honors, Awards, and Publications

Publications other than abstracts from this project:

Andrews, J. R., Swarm, R. L., Schlachter, L., Bruce, I. C., Rubin, P., Bergenstal, D. M., Gump, M., Siegel, S., and Swain, R. W., The Effects of One Curie of Sulfur-35 Administered Intravenously as Sulfate to a Man with Advanced Chondrosarcoma. This paper was accepted for publication in the Am. J. of Roentgenol. and Radium Therapy in 1958 and the galley proof has been received but for reasons unknown to me it has not been published.

Honors and Awards relating to this project:

None

2. Rad. Ther. Serv.
3. Bethesda, Md.

Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Clinical Radiation Dosimetry

Principal Investigator:

Robert W. Swain

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: To develop methods for and to determine radiation dosage distributions for various types of radiation therapy used in the treatment of patients and to devise methods for improvement of radiation distribution within the patient.

Methods Employed: Chemical and ionization chambers for cobalt beam therapy. Ionization chambers and photographic methods for Supervoltage X-ray Therapy.

Major Findings: Work on the isodose plotter had been stopped due to a shortage of personnel. At the present time, all electrical and mechanical components have been assembled for semi-automatic plotting of isodose distributions in water using a small ionization chamber. As time permits, trial runs will be made to determine desirable modifications.

To improve dosage distributions for treatment of patients, wedge filters have been developed for use on the 2 Mev Van de Graaff generators. Preliminary dosage distributions were made using film dosimetry methods. The studies made indicate a possible inaccuracy in the film method which will be investigated when the plotter is completed.

Project Description: (Continued)

The dosimeters in the 2 Hew Van de Graaff Generators are being replaced by transmission ionization chambers connected to current integrators which can be preset to turn the x-rays off at the end of a prescribed dose. This should lead to greater accuracy of treatment and reduce possibility of overexposure since at the present time, the technician is required to turn off the generator when a meter indicates the prescribed dose.

Proposed Course of Project: Work on the isodose plotter will be continued with the objective of making it as accurate as possible and as completely automatic as practical.

With the availability of the 256-Channel analyzer, a project will be undertaken to determine x-ray spectra.

Search will be continued for small ionization chambers or other types of dosimeters to facilitate assessment of dosage during treatment of patients.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

An Appraisal of Renal Function and Infection in Advanced Bladder Malignancy Treated with Ileal Bladder Construction and X-Irradiation.

Principal Investigator:

J. Robert Andrews, M. D.

Other Investigators:

Jack Levin, M. D., Herman D. Suit, M. D.

Cooperating Units:

None

Man Years (calendar year 1959)

Total:
Professional:
Other:

Patient Days (calendar year

Project Description:

Objectives: Evaluation of the results of urinary stream diversion and radiation therapy for advanced carcinoma of the bladder, and an appraisal of renal function and infection in these cases.

Methods employed: Patients with Stage C and D carcinoma of the bladder, grade 3 and 4, had urinary stream diversion by construction of an ileal bladder (surgery performed by the Surgical Branch of the National Cancer Institute), and subsequently had high doses (7000 to 8000 r in 7 weeks) of 8 Mev x-ray therapy delivered to the bladder and the surrounding pelvic structures.

Patient Material: Nine patients have been so treated. These cases and 2 patients who have had diversion of their urinary stream following x-ray therapy have constituted the source for the study.

Major Findings:

A. The combination of diversion of the urinary stream and irradiation to the pelvis has resulted in marked symptomatic improvement as manifested by control of dysuria, frequency, urinary tract obstruction, and hematuria. There has been at least temporary control of tumor following irradiation. However, 9 of the 11 patients are now dead, 7 of the 9 with distant metastases. The two living patients have survived 25 and 34 months respectively.

B. Renal function and bacteriological studies.
Renal function has been studied in these patients by means of the blood urea nitrogen, serum creatinine, creatinine clearance, and fractional PSP excretion. Infection of the urinary tract has been assessed by the use of quantitative urine cultures. Following diversion of the urinary stream and treatment with irradiation, renal function as measured by the blood urea nitrogen, serum creatinine, and IVP examination indicated a prompt and striking return to normal levels. However, the endogenous creatinine clearance and fractional PSP excretion did not show any such improvement. On the contrary renal function as measured by these tests was markedly depressed. This surprising divergence of data is as yet unexplained.

There was no significant change in the percentage of positive quantitative urine cultures post-operatively. Presumably, therefore, infection of the upper urinary tract is not eradicated by urinary stream diversion. In these patients the absence of clinically acute pyelonephritis does not rule out the presence of a silent infectious process occurring in the kidneys.

Although none of our patients demonstrated evidence of urinary tract obstruction following urinary stream diversion our data suggests to us that damage to the kidneys from prolonged urinary tract infection and ureteral obstruction is not necessarily corrected by relief of urinary tract obstruction, and the extent of bacteriuria remains unaltered. We have concluded that our patients remain in a marginal status with respect to renal function despite the apparent benefit indicated by the serum creatinine, BUN, and IVP. The procedure was, however, of real symptomatic benefit to the patient and greatly facilitated radiation therapy.

It is suggested that renal function is more compromised in these patients than appreciated previously, and that further study is needed to evaluate the long term effects and benefits from urinary stream diversion and irradiation in cancer of the bladder.

Part B included Yes ☐ No ☐

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Radioactive Isotopes in Interstitial Radiation Therapy

Principal Investigator:

Herman D. Suit, M. D.

Other Investigators:

Robert W. Swain and Calvin R. Meneken

Cooperating Units:

None

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total:
Professional:
Other:

Project Description:

Objectives: (1) To develop a technique and equipment for placing small radioactive sources, as a permanent implant, in inaccessible anatomical regions, so as to deliver a high total dose locally but a low dose to the operator and other medical and nursing staff. (2) To develop technique and equipment for achieving dose distributions comparable to that obtained with radium needle therapy but with greater flexibility as to dose distribution and with reduced radiation exposure to operator and staff.

Methods Employed and Major Findings: This was described in detail in the individual project report of calendar year 1958.

Proposed Course of Project: The magazine loading radioactive seed implanting device has been loaned to Dr. Gilbert Fletcher, M. D. Anderson Hospital, to use in a controlled study of cancer of the urinary bladder. Cases will be treated on a random basis using external beam radiation therapy in both groups and

Project Description: (continued)

supplementary radioactivity implantation therapy in one group. The M. D. Anderson Hospital has the patient material and the other resources to conduct an extensive trial of the instrument.

Part B included Yes ☑ No ☐

Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

None

Honors and Awards relating to this project:

An exhibit on this material was prepared and shown at the Annual Meetings of the American Roentgen Ray Society in Cincinnati and at the Annual Meeting of the Radiological Society of North America in Chicago where it received a citation.

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1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Studies on Thyroid Cancer

Principal Investigator:

J. Robert Andrews, M. D.

Other Investigators:

Theodore Robinson, M. D., James Rose, M. D.

Cooperating Units:

None

Man Years (calendar year 1959): Patient Days (calendar year 1959):
Total:
Professional:
Other:

Project Description:

Objectives and Methods Employed: To study the factors influencing the response of carcinoma of the thyroid gland to radioactive iodine in association with thyroid hormone treatment. To study the turnover rates of primary carcinoma of the thyroid and metastases and their response to hormonal and other factors. To study the hormone effect, and hyperthyroidism on thyroid tumors. This study involves a variety of considerations including technicological, biological, endocrinological and pharmacological aspects. Technical studies are concerned with problems associated with the identification and evaluation of radioactivity in specific organs or anatomical sites. Biological studies are concerned with the relationship of the histopathological characteristics of thyroid cancer to radioactivity uptake and response. Hormonal considerations are concerned with the relationship of tumor control to the hormonal status of the individual alone

Project Description: (Continued)

and in combination with radioactive iodine. Also being studied is the relationship of turnover rates (of RAI-131) of thyroid tumor in relationship to the ingestion of thyroid products and other substances to see if radioactive substances may be held in tumor tissue for a longer period, thus increasing the amount of radiation to the tissue.

Patient Material: Proved cases of carcinoma of the thyroid, either primary or secondary or both.

Major Findings: Findings from this study seem to show that the use of radioactive iodine along with thyroid hormone seems to have a tumoricidal effect beyond that of mere radioactivity alone. Due to the fairly recent origin of this combined technique equivalent studies to which our results could be compared are not readily available. Results also seem to show that mere thyroid hormone alone may have some effect on causing regression on thyroid tumor. Work with small animals seems to show that administration of thyroid hormone causing the animal to retain the hyperthyroid state increases the radiation response. It also seems to increase the radiation response to tumor within the animal and possibly hyperthyroidism alone in one form of tumor may have a somewhat slowing effect on the growth of the tumor. Results are questionable as to whether or not turnover rates of functioning thyroid tumor may be demonstrably altered by the use of endocrinological agents.

Proposed Course of Project: To continue. In addition to continuing those observations which are described above a study of hemagglutinating autoimmune antibodies in response to thyroglobulin and tumor fractions is also being undertaken.

Part B included Yes ☐ No ☒

[redacted]

FWS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Radiation Induced Dysplasias of Bone

Principal Investigator:

Philip Rubin, M. D.

Other Investigators:

Hazel Gump, A. B., Richard Swarm, M. D., and J. Robert Andrews, M. D.

Cooperating Units:

Strong Memorial Hospital, Department of Radiology, University of Rochester, Rochester, New York

Man Years:
Total:
Professional:
Other:

Project Description:

Objectives and Methods Employed: This project concerned the induction of dysplasias of bone by irradiation with either internal emitters or external x-irradiation. The internal emitters employed were S-35 and Sr-90. S-35 affects largely epiphysial growth and metaphysial change while Sr-90 affects largely diaphysial growth. External irradiation was given selectively to either an entire bone, the epiphysis, the metaphysis or the diaphysis. Novel means for precise local irradiation of the experimental animal were developed.

Major Findings: It was found that local irradiation of portions of growing bone in appropriate doses caused characteristic deformities of the bone. These deformities were related to certain biomechanical aspects of bone modeling and to the clinical osteochondrodystrophies. On theoretical bases good correlation could be shown between the experimentally induced radiation dysplasias and the characteristics of the clinical osteochondrodystrophies of unknown cause. The results of this study have been published.

Proposed Course of Project: This project was brought to completion. The University of Rochester studies commenced by Dr. Philip Rubin and others while Dr. Rubin was at the National Cancer Institute.

Part B included: Yes ☒ No ☐

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Rubin, P., Andrews, J. R., Swarm, R. and Gump, H., Radiation Induced Dysplasias of Bone, American Journal of Roentgenology and Radium Therapy, 82: 206, 1959.

Honors and Awards Relating to this Project:

None

1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Development of Wedge Filters for a 2 Mev X-ray Beam

Principal Investigator:

Stanley E. Sneider, M. D.

Other Investigators:

Herman D. Suit, M.D., Robert W. Swain and J. Robert Andrews, M.D.

Cooperating Units:

None

Man Years (calendar year 1959)
Total:
Professional:
Other:

Project Description:

Objectives: The isodose curves used in conventional radiation therapy lie flat across the field, that is, they are fairly parallel with the surface being treated. We have undertaken to change the shape of the isodose curves produced in a beam of x-rays from a 2 Mev Van de Graaff accelerator so that the curves form an acute instead of a right angle with the central axis of the beam. The idealized curves would begin at one edge of the field at a point and ascend at a given angle (45° or 60°) in a straight line across the width of the field. This distortion of the radiation distribution in a field is useful when two or more fields are combined in the treatment techniques for certain anatomical sites in sharply diminishing the radiation given outside of the volume of the disease bearing tissue and in partially or completely avoiding structures which are sensitive to high dose irradiation. Although wedge filters have been in clinical use in a few radiation therapy centers for 20 years, we planned to study wedge design and composition (effect of atomic number) in an attempt to produce "idealized" angling of the isodose surface.

Project Description: (Continued)

In addition to the design of the wedge filters themselves we have been concerned with (1) the possibility of using just two wedged filters for specified angled isodose curve (e.g. 45°) over a range of clinically useful field sizes; (2) the employment of lucite bolus material at the machine head to compensate for air gaps when the x-ray beam enters the surface of a patient obliquely; and (3) the evaluation of this treatment technique in actual case material.

Methods Employed: On a set of standard isodose curves lines were drawn at 45° or 60° to the central axis of the field. These lines started at one edge of the field and ascended across the field. The starting point is taken to be at the surface edge projected into the phantom rather than the geometric edge. The angled line is planned to have a single isodose value and this value is taken to be the value of the standard isodose curve which the newly drawn angled line intersects at the field edge. At various points along this angled line differences in percentages of depth dose between the value of the angled line and the regular isodose curves are determined. The amount of lead required to achieve this reduction in the depth dose is then read from an absorption curve for the 2 Mev beam in lead. This absorption curve was determined experimentally for our machines using a 10 x 10 cm. field. This procedure then gives the height of lead at various points across the wedge to effect the desired dose reduction.

The width of the wedge is then adjusted to 4.8 cm. so that it can be placed at the machine head rather than at the patient's surface. A piece of pure lead was then machined to have the contour calculated.

Kodak positive grain film was sealed in a masonite phantom, 25 x 25 cm., and exposed to the 2 Mev x-ray beam. A number of exposures of this type were carried out with and without the wedge filter in the beam. The films exposed without the wedge served as controls. Controls and wedged films were developed simultaneously and in similar manner. As the blackening of the film is related to the radiation dose a densitometer was used to estimate the shape of the isodose curves.

This 4.8 cm. wedge permitted a range of field sizes from 7 x 7 cm. to 14 x 14 cm.; a wedge 3 cm. wide was then milled having the same absolute lead thickness of the 4.8 cm. wedge. This 3 cm. wedge permitted a range of field sizes from 4.5 x 4.5 cm. to 8.5 x 8.5 cm. The smaller one can be used for small fields and the larger one for large fields by simply moving the wedge closer to or further from the machine head. The validity of this concept was checked by the film procedure mentioned above.

Project Description: (Continued)

When the incident x-ray beam enters a surface obliquely one edge enters the absorbing media before the other. Because of this the isodose curves are not similar to those obtained when the beam is normal to the surface. To compensate for the lack of absorbing media in part of the beam a wedged piece of lucite bolus was made to fit with or without the wedge filter at the machine head. The film technique was used to check on the resulting isodose curves when the lucite bolus was used for wedged oblique fields and when it was not.

A case of carcinoma of the right vallecula and glosso-pharyngeal sulcus was used as a practical application study. Film was sealed in a shaped like a head and neck and exposed in the same treatment set-up, using obliqued wedged fields, as was the patient. This film was scanned as mentioned.

A total of three wedges were constructed (two for 45° and one for 60°) and evaluated as indicated.

Major Findings: The angles of the produced isodose curves were nearly at the predicted values. A discrepancy in the depths of particular curves was noted, however. It is thought that the response of the film used is dependent upon the energy of the x-rays to which it is exposed if the x-rays are less energetic than 200 KV. Because of the discrepancy in depths of the curves it is believed that the scattered radiation within the phantom contains appreciable quantities of x-rays less energetic than 200 KV. Though the curves were nearly at the desired angles, the deviation of the curves from the predicted value was in the direction that would occur if the discrepancy were due in large part to the increased response of the film to the less energetic x-rays.

The concept of using two wedge filters for a range of field sizes from 4.5 x 4.5 to 14 x 14 cm. proved to be accurate from the film analyses. Likewise the use of lucite bolus proved to be helpful in compensating for the air gaps made when the beam enters obliquely. Study of the particular case in question showed that the volume desired to be treated had a fairly uniform dose and that outside of this volume the dose was no more than half.

Significance to Cancer Research: The development of wedge filters specifically for use here will extend the range of techniques available to the radiotherapist. In selected cases, treatment planning will be easier in that in comparison to conventional techniques this technique will allow a smaller number of fields, reduction of the dose delivered to tissues outside of the area being treated and partial or complete avoidance of sensitive structures.

Project Description: (Continued)

Proposed Course of Project: At present a scanner using a moving ionization chamber in a water phantom as a recorder of dose is under construction. It is planned that the ionization chamber will be independent of the energy of the x-rays to which it is exposed over the range of interest to us. It is planned to re-check the results of the film technique with this chamber scanner. In addition more specific tumor situations will then be evaluated and studies concerned with the use of different metals besides lead will be possible.

Part B included Yes ☐ No ☒

Serial No. NC1-696

1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Studies on Bronchogenic Carcinoma; Comparison of Combinations of Chemotherapy and X-Ray with X-Ray Alone

Principal Investigator:

J. Robert Andrews, M. D.

Other Investigators:

Kirkland C. Brace, M. D., C. Brindley, M. D., Gordon Sharp, M. D., and Louis V. Avioli, M. D.

Cooperating Units:

Lemuel Shattuck Hospital
D. C. General Hospital
Johns Hopkins Hospital

Man Years (calendar year 1959): Patient Days (calendar year 19[illegible])
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: Since most, if not all, of the investigative approaches comparing the effect of x-ray alone with x-ray combined with nitrogen mustard therapy as possible treatment for bronchogenic carcinoma, have been retroactive [illegible], this project has been established to permit the complete randomization of selection essential for such an analysis.

Methods: All patients will receive radiation therapy during the course of treatment and are to be randomly divided into three groups:

1.) Those receiving radiation alone.
2.) Those receiving mustards before radiation.
3.) Those receiving mustards during radiation.

Project Description: (Continued)

Patient Material: Patients will be included in the study when they have had:

1.) Biopsy proof of inoperable or recurrent bronchogenic carcinoma.
2.) Any previous treatment with x-ray, or alkylating agents must have been concluded at least six weeks before the start of the study.
3.) Existence of a unilateral pulmonary lesion visible by x-ray.

Major Findings: The study has not been completely evaluated as yet but every patient treated with X-radiation to date has evidenced some regression of the neoplastic process. The series has not been prolonged enough to appreciate any additive effects of nitrogen mustards at the date of this writing.

Proposed Course of Project: To continue.

Part B included Yes ☐ No ☒

Serial No. NCI-69%
1. Radiation Branch
2. Rad. Ther. Serv.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

"Oxygen Effect" and Tumor Size in Local X-irradiation of the C_3H/BA Mammary Adenocarcinoma.

Principal Investigator:

Herman D. Suit, M. D.

Other Investigator:

Lawrence Schlachter, M. D.

Cooperating Units:

None

Man Years (calendar year 1959)
Total:
Professional:
Other:

Project Description:

Objectives: To determine the effect of increased oxygen tension and initial tumor size on the local response of the C_3H/BA Transplantable adenocarcinoma after x-irradiation.

Methods Employed: The C_3H/BA adenocarcinoma was transplanted intramuscularly in the thighs of C3H Heston strain mice, and the response of this tumor was measured using local cure as the criterion of measurement. Anaimals bearing tumors with a mean diameter of 1 cm. and animals bearing tumors with a mean diameter of 2 cm. were irradiated in separate groups, half of each group being irradiated under air at one atmosphere and the other half under 100% oxygen at two atmospheres of pressure.

Major Findings: There was found no statistically significant difference in the local response of the tumors in those animals treated with 100% oxygen at two atmospheres and those animals treated with air at one atmosphere but a statistically significant increase in local cure was obtained when those animals bearing small tumors were irradiated as contrasted to those animals bearing large tumors.

Project Description: (Continued)

Significance to Cancer Research: Improvement in clinical radiotherapy.

Proposed Course of Project: To be discontinued.

Part B included Yes ☐ No ☒

Serial No. NCI-10-750 (c
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Removal of Cancer and Other Tissues for Histologic, Biochemical and Other Studies as Required by Scientists and Other Investigators for Correlated Studies.

Principal Investigator: Dr. Robert R. Smith

Other Investigators: Dr. Alfred S. Ketchum and Dr. John F. Potter

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1959):

Project Description:

Objectives:

The object of this project is to supply human tissue for histologic, histochemical, biochemical and biophysical studies to scientists working in various branches of the National Cancer Institute, who require tissue for studies. In addition, it implies furnishing general surgical coverage of patients admitted to other branches of the N.C.I. and, or other institutes, and assisting with their study projects. As a rule, such studies are done as cooperative projects with various investigators but on occasions, specific types of tissue are required for specific studies. For example, one investigator requested that specimens of skin and its associated neoplasm be made available for keratinization studies, and various muscle biopsies and, or biopsies of liver tissue under specific conditions have been requested. These materials are usually obtained during operations for standard therapeutic reasons.

Methods Employed:

Methods employed are standard surgical procedures.

Patient Material:

Material for this project is obtained mostly from patients

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hospitalized for study in other projects in other branches or institutes. Occasionally, it is necessary to admit patients for specific studies in which a specific type of tissue is obtained. There have been several examples of this, mostly at the request of the dermatologist who wanted large volumes of basal cell carcinomas for a specific study. Even in these studies, data for other projects were obtained; for example, the wound seeding study and another example is a beryllium granuloma. On several occasions patients were admitted by other institutes for material for special *in vitro* studies (mostly thyroid adenomas) and patients with obstructive jaundice in order to obtain bile for biochemical studies. It is difficult from the data available to determine just which cases were admitted for specific studies. There were 171 minor operations done to obtain tissue for biopsy, and, or biochemical, biophysical, and histochemical studies. In addition, there were 57 major operative procedures performed for other investigators. This surgery work load was derived from answering 643 requests for consultation.

Major Findings:

Presented in reports of other projects by other investigators.

Significance to Cancer Research:

This project is more or less a service function for other branches of the National Cancer Institute and the National Institutes of Health.

Proposed Course of Project:

The number of such cases seems to be increasing as demand for assistance in obtaining tissue for special study increases.

Part B included Yes [ ] No [XX]

Serial No. MCI-10-751(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Evaluation of Radical Surgery and the Development of New Surgical Techniques as a Therapeutic Means of Palliative or Definitive Therapy of Cancer.

Principal Investigator: Dr. Robert R. Smith

Other Investigators: Dr. Alfred Kotchom, Dr. John Potter, Dr. John Dillon, Dr. Robert Hoye, Dr. Marvin Romsdahl, Dr. Howard Radwin and Dr. Jack Bloch

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1-3/4
Professional: 1½
Other ¼

Patient Days (calendar year 1959):

Project Description:

Objectives:

The objectives of this project are to develop and improve methods of obtaining increased palliation and/or definitive cure of cancer in man. In addition, the study attempts to evaluate the rehabilitation status of patients undergoing radical surgery.

Methods Employed:

This project is made up of a number of separate parts, with clinical and laboratory components. The clinical part consists of evaluation of radical surgery, mostly head and neck and pelvic cancer patients. These patients have proven cancer with no clinical evidence of spread beyond the operative area. The study involves review of specific types of case records which now are beginning to accumulate in numbers which allow some analysis of method and results.

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Cervix Cancer - Patients with more advanced carcinoma of the cervix uteri, without evidence of distant spread, are admitted for study. These patients receive as complete a work-up as possible and are followed in a continuing study to determine the biologic behavior of this type of cancer influenced by various treatment regimens. The cases that are operable receive excisional surgery. A total pelvic exenteration consists of removal of the contents of the pelvis, including the rectum, uterus, adnexa, vagina, urinary bladder, and all pelvic lymph nodes. An anterior exenteration is the same except the rectum remains intact. The important change in operative procedures is the use of an ileal conduit in place of a wet colostomy in cases in which it is necessary to remove the urinary bladder.

An attempt is being made to correlate the various parameters used in the preoperative work-up of patients admitted to the Clinical Center with carcinoma of the cervix with the findings at surgery, in the pathology specimen, and at postmortem. An attempt will be made to evaluate the prognostic significance of the various preoperative tests and both surgical and pathologic findings. A careful review of the postoperative course of all patients will be made to evaluate our current methods of follow-up and to compare to some extent the courses of operable and inoperable patients and the efficacy of radical surgery in treating carcinoma of the cervix.

The evaluation of the ileal loop involves the correlation of the clinical course of the individual patient with objective measurements of renal function, x-ray studies and urine cultures. Particular attention is given to the ability of the ileal bladder to prevent progressive renal failure, which has often already commenced at the time of surgery. All patients for whom ileal loops have been created prior to July 1, 1959, are included in the study. These consist primarily of women with carcinoma of the cervix together with a much smaller number of patients with carcinoma of the urinary bladder.

Head and Neck Cancer - Patients with primary cancer of the head and neck area are admitted for complete work-up. Of special interest to this project is the group of paranasal sinus cancers. These cases are subjected to radical excision by a combined neuro-surgical approach. This procedure has been described previously. Very few patients with this type cancer are seen at any time, which requires that the study be continued over a long period of time.

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Dr. Robert Hoye is analyzing the group of head and neck cancer patients who have had an autopsy performed, and Dr. Marvin Romsdahl is reviewing cases of carcinoma of the larynx and pharynx. These cases are reviewed with the intent of determining the results of different types of therapy, changes in the biology of the cancer produced or associated with therapy, and the social and economic rehabilitation of this group of patients. Patients will be included in the rehabilitation study who are at least six months postoperative and without evidence of recurrent disease at the time the evaluation is made. The four areas for evaluation include: (1) activities of daily living, (2) activities of family life, (3) activities of community life, and (4) earning ability and employment. Each patient will be evaluated by interview and given a grade both preoperatively (prior to onset of disease) and postoperatively. These grades will then be tabled in each specific category from all patients studied and comparisons determined between preoperative status and postoperative status. The difference will then be indicative of adjustment in each specific category.

Patient Material:

Cervix Cancer - Approximately 180 patients with cancer of the uterine cervix have been seen since the opening of the Clinical Center. A majority of these received some form of definitive surgery (± 85%). Most have advanced cancer and ±50% have had previous radiation therapy. Many of these patients were also studied in the virus therapy program. Those patients that have had exenterative surgery provide material for the ileal bladder study (± 60 pts.).

Approximately 100 patients with invasive intra-oral and/or pharyngeal-laryngeal cancer have received definitive surgical therapy. Twenty-five of these have come to autopsy and provide material for special study of the biologic behavior of this type of cancer influenced by surgical therapy. About 40 patients that have had radical excisions of the head and neck area have returned home at least six months ago and are now free of disease and are available for study as to the social and economic rehabilitation they have made.

Major Findings:

Cervix Cancer Group - A complete review of this group of cases is under way and will be available for study in the next few months. In general, it appears that exenterative surgery does have a worthwhile place in the treatment of carcinoma of the uterine cervix.

We have learned that social rehabilitation and acceptance of these patients by their family and friends remains good as long as they remain free of their cancer. The colostomy and/or ileostomy management depends upon the sincerity and diligence of the physician-nurse training given the patient. The procedure has become accepted in the community as demonstrated by the referral of suitable patients to our service by the professors of gynecology of the local medical schools as well as by most of the gynecologists in the area.

The ileal bladder appears to be a satisfactory method of urinary diversion in the cancer patient. Ascending infection and hyperchloremic acidosis are unusual when this technique is compared with the previously employed method of wet colostomy. Roentgenologic studies indicate improvement in renal function in almost half the patients. Deterioration is rare in the absence of recurrent tumor. Still to be interpreted is the significance of the presence of ureteral reflux together with continued bacterial contamination in a large number of patients. The most worthwhile factor observed in the ileal bladder group is the marked improvement noted in the case of nurse-patient management. As the study progresses it becomes more apparent that this method of urinary diversion is inherently sound and that the technical problems of the surgery depend upon the ability of the surgeon. When failure has occurred, it was found dependent upon regrowth or extension of the original cancer.

Head and Neck Cancer Group - We have thus far reviewed 25 patients who satisfy the above criteria. Twelve different primary sites are represented and approximately equally distributed between those patients with distant metastases and those without. Thirteen patients have demonstrated spread below the clavicle to 17 different organ systems. The lung contained metastatic tumor in 12 of the 13 cases and this incidence differs quite markedly from the 12.7% incidence of lung involvement in the 66 patients with distant metastases reported by Braund and Martin in 1941. Three of our patients with distant spread did not have any cervical lymph node involvement and in none was there invasion of the internal jugular vein. The only patient with internal jugular vein invasion died seven months later without evidence of distant spread. In the series reported by Braund and Martin the interval from the time of primary treatment to death was the same in those with and those without distant spread and averaged eight months. In our series this interval was nineteen months in the group with distant spread as compared to ten months in those without distant spread. In the group having distant metastases every patient living longer than three months following primary treatment developed and was treated for at least one local recurrence of his tumor. In all there were 28 recurrences in this group as compared

to 6 in the group not demonstrating spread below the clavicles. In the 13 patients with distant metastases, 11 had evidence of local disease at autopsy, the 2 not demonstrating local disease at autopsy had recurrences excised seven and twelve months previously.

When the histologic type of tumor was broken down into anaplastic, moderately well differentiated, and well differentiated, there was no evident correlation among the two groups.

For any therapeutic measure to be accepted as worthwhile, it must be safe and produce improvement in the general well-being of the patient that allows them to lead a useful life within the limitations of their preoperative status. The effectiveness of any surgical procedure is measured by the risk of operation compared with the risk of the disease. During the past year studies have been undertaken to evaluate the rehabilitation status of patients treated 2-5 years ago. Detailed results of these studies are not available. From individual experience, however, it is believed that patients with advanced cancer can be rehabilitated after radical surgical excisional therapy, and that even in cases where palliation only is possible, worthwhile results can be obtained. A statistical evaluation of these results will be available in six months.

A study of surgical complications for the year September 1958 - 1959 has been completed. The results show that at NCI the complication rate is well within acceptable limits and that operative mortality is very low. During this period, 245 major and 214 minor surgical excisions were performed. There were 7 deaths occurring within the 30-day postoperative period. Three of these occurred on 10-East and are legitimate operative deaths. They were due to (1) an acute myocardial infection occurring ten days after an exenteration of rectum and bladder, (2) an exsanguinating hemorrhage from the carotid artery seven days after a laryngectomy and neck dissection, and (3) an unresolved death associated with irreversible shock occurring five days after exploration for intestinal obstruction occurring one month after total pelvic exenteration for advanced carcinoma of the cervix. Autopsy, still incomplete, showed thrombosis of the lower vena cava but no anatomic changes that could have unequivocally caused her death.

The remaining four deaths were as follows:

(1) A patient died of hepatic failure due to acute infectious hepatitis occurring in a patient with cirrhosis of the liver. Death occurred a few days after exploration to rule out any possible

extra-hepatic obstruction that could be relieved. None was found.

(2) A patient with macroglobulinemia died two days after exploratory laparotomy with signs of acute peritonitis. None was found. She did have an infarcted spleen.

(3) A choriocarcinoma patient died of hemorrhage six days after a "last ditch" effort to control hemorrhage from tumor that extened to the lateral pelvic wall.

(4) A Radiotherapy patient with carcinoma of the tongue developed a perforated colon with generalised peritonitis. One month later massive gastrointestinal bleeding required a subtotal gastric resection to remove gastric and duodenal ulcer. On the seventh post-operative day he died with a septicemia due to hemolytic Staph aureus.

It is the writer's personal opinion that in the last four cases surgery was only incidental to the factors that caused their deaths. The remaining three deaths in 245 major operations gives an operative mortality rate of 1.1%, a reasonable value considering the magnitude of the surgery involved.

There were 44 infections occurring in the 245 major cases, giving a crude infection rate of 17.9%. If the data is calculated upon clean cases only, the infection rate was 2.4%. If contaminated cases are included (i.e. those with preoperative cultures of pathogens from secretions or those whose bowel or oropharynx opened into the operative field) but excluding dirty cases (those who at the time of surgery had a documented infection) the corrected infection rate was 13.9%. A majority of our cases fit into this group.

When one tries to determine possible causes for the infections observed, three factors appear:

(1) Avascularity of the operative wounds. - This was present in 20 of the 44 cases (i.e. skin flaps in radical breast amputation or in groin dissections). It is associated frequently with extensive pre-operative radiation, and in many instances, a necessary factor of cancer surgery.

(2) The presence of antibiotic resistant coagulase positive hemolytic Staph aureus organisms found in 29 of the 44 cases. It is interesting to note that an additional 17 patients entered the operating room with recurrent or persistent infection with this organism, but did not develop subsequent postoperative infections.

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(3) Contaminated wounds as a major factor in infection was seen in 19 instances. These cases include those in which the mouth, pharynx, trachea, or gastrointestinal tract opened into the wound, or known contamination by preoperative cultures of skin or secretions draining into the operative field. It was noted that in 4 of these cases there was significant avascularity and in 9 there was continued bathing of the wound in contaminated sputum.

The very nature of most cancer surgery determines that many wounds will be contaminated. The problem therefore is one of learning how to manage these patients so that the body defense mechanisms can be strengthened so that wounds will heal and generalized infection not develop.

On the basis of this retrospective study of operative complications, a perspective study is underway to measure the effectiveness of prophylactic antibiotic therapy in contaminated cases. Our experience with present methods has shown that the antibiotics used in the past were of no value, for the organisms cultured from these wounds were resistant to the antibiotics used. There was no indication from the study that the operating rooms or our wards were the source of infection. In fact, the problem lies in the infection the patient takes with him to the operating room. This was proven in 23 of 30 patients studied.

The continuing study of operative mortality shows that when surgery is to be used as a last measure, the mortality rate will be high. On the other hand, radical surgery can be effectively used in treatment of advanced cancer and give a low operative mortality rate if adequate pre- and postoperative care is given and if the surgeons involved are given the support they require. This type of surgery can carry a prohibitive operative mortality if the surgeon does not have the opportunity to gradually obtain experience in this type of case.

Significance to Cancer Research:

A study of carcinoma of the cervix is underway at several large centers where radical surgery is being used. The value of this study lies not so much in the efficacy of radical surgery but in the fact that our patients have been worked up preoperatively and pathologically and followed in great detail so that the behaviour of carcinoma of the cervix and alterations in the biological behaviour associated with radical surgery can be studied. In addition, the ileal bladder is an important adjunct to the ablative procedures employed in cancer surgery. Its rationale lies in the necessity of providing a replacement for an organ removed because of involvement by tumor.

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It appears that the patient with epidermoid carcinoma of the upper respiratory or alimentary tract who is most likely to develop spread of his disease below the clavicle is one in whom control of the primary lesion has been a significant problem; one who develops and is treated for at least one local recurrence of his tumor and whose life span from the time of primary treatment has been prolonged by repeated attempts at cure. These findings suggest that the longer a focus of carcinoma remains at the primary site the greater will be the incidence of distant metastases.

We have shown that distant spread can occur without involvement of the cervical nodes or internal jugular vein, and that the most frequent metastatic site is the lung. Radical cancer surgery has become a tool aimed at increasing the cancer salvage rate. The use of this tool brings other factors into play other than the cure rate or longevity of life. We must be concerned with whether or not a useful and adjusted citizen is returned to society following these radical procedures. By necessity, this evaluation should be understood to concur justification regarding this type of therapy. This study is aimed at this particular group in an attempt to objectively evaluate their adjustment or rehabilitation.

Proposed Course of Project:

In the future we plan to expand our series of cases by including those patients treated in the Clinical Center and subsequently dying away from the hospital and coming to autopsy.

The size of the primary lesion, the pathological report on the margins of the resection for tumor, the incidence of positive wound washings, and the rapidity of onset of the first recurrence are factors which have not been correlated in the present study with the frequency of distant metastases and will be incorporated in further work. Also the series will be expanded to include all patients treated at the Clinical Center for carcinoma of specific area, so that comparisons between living and autopsied patients can be made.

It is hoped that this continuing study will enable one to identify the biological characteristic of specific cancers which will point to early classification of which tumors will spread to distant sites, and the manner in which this dissemination occurs.

Part B included: Yes [XX] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Lusted, Lee B., Ship, Arthur, Smith, Robert R. and Jude, James R.: Postoperative Investigation of the Ileal Bladder. Radiol. 72:2, 211-216, 1959.

Jude, James R., Harris, Alvin H. and Smith, Robert R.: The Physiologic Response to the Ileal Bladder. Surg. Gynec. & Obst. 109:2, 173-176, 1959.

Cramer, Lester M., Waite, John H., Edgcomb, John H., Powell, Clinton [illegible] Treuhy, John H. and Van Scott, Eugene J.: Burn Following Accidental Exposure to High Energy Radiation. Ann. Surg. 149:2, 286-293, 19[illegible]

Jude, James R., Lusted, Lee B. and Smith, Robert R.: Radiographic Evaluation of the Urinary Tract Following Urinary Diversion to an Ileal Bladder. Cancer 12:6, 1134-1141, 1959.

Honors and Awards relating to this project: None

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1. Surgery Branch
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Part A.

Project Title: Part I. The Virus Treatment of Human Carcinoma of the Cervix.

Part II. Pyridoxine Deficiency in Conjunction with Virus Treatment of Cervix Cancer.

Principal Investigator: Part I. Dr. Robert R. Smith, Dr. Robert J. Huebner, Dr. Robert B. Couch, Dr. Robert A. Manaker, Dr. Robert Love, and Dr. Wallace Rowe

Part II. Dr. Robert B. Couch

Other Investigators: Part II. Dr. Robert R. Smith, Dr. Edward McLaughlin, Dr. Robert Manaker, Dr. Helen Dyer, and Dr. Robert Crounse

Cooperating Units: Part I. Laboratory of Biology, NCI; Laboratory of Pathology, NCI; and Laboratory of Infectious Diseases, NIAID

Man Years (calendar year 1959):
Total: 1½
Professional: 1
Other: ½

Patient Days (calendar year 1959):

Project Description:

This project is made up of two parts, and these will be presented separately.

Objectives:

Part I.

In the introduction of viruses associated with human tumors project, the theoretical associations that may occur between viruses and tumors are presented. A cooperative project between the laboratories listed above has been underway for 5 years. This project involves the study of the effects of artificially infecting human tumors with viruses. Previous annual reports have presented the yearly progress and the publications resulting. Studies with the adenoviruses with and without concomitant administration of cortisone resulted in encouraging therapeutic responses. In an

effort to enhance the resultant oncolysis, a serial study was undertaken in the clinical situation utilizing Coxsackie B3 virus. This study is based on the demonstration by Suskind, et al, that passage of virus tumor to tumor in a rat tumor system resulted in enhanced oncolytic effect. This report will be a summary of the Coxsackie phase of this human treatment study. The current investigation is directed toward detailed study of the patients in an effort to see if passage has brought about any change in tumor response to virus and behavior of virus in this artificial situation.

Part II.

In the virus treatment of cancer there is an apparent relationship between frequency of antibody formation and frequency of recovery of virus from the injected tumor. It is not known to what extent serum antibody, as opposed to other mechanisms of host resistance to infections, influences virus survival. However, it was felt that if antibody formation could be altered, then perhaps virus survival would be prolonged and degree of tumor oncolysis be increased.

In a search of the literature as regards possible means of temporarily inhibiting host resistance to infection, attention was drawn to the deficiency states. It has been shown that pyridoxine, pantothenic acid, and folic acid deficiency in rats results in inhibition of antibody formation. Previous studies in the surgical branch with pantothenic acid deficiency were unsuccessful in this respect, but probably a deficiency state was never achieved. In view of the availability of a potent antagonist to pyridoxine, induction of this deficiency in the human seemed possible. Accordingly, a protocol was designed to study the effect of pyridoxine deficiency on antibody formation, virus survival, and tumor oncolysis in the human host. Studies were included to clarify the nature of human pyridoxine deficiency and measure the degree of deficiency state. This study is in progress, and this constitutes an interim report.

Methods Employed:

Part I.

Two methods of treatment and study have been employed. The first method consisted of a 14 day isolation protocol. The virus was injected directly intra-tumor on day 1. Virus cultures were made of vaginal discharge and tumor biopsies on days 0, 2, 4, 6, 8, 10, and 14. Serum was collected simultaneously for antibody titers. Observation for tumor necrosis was made at the time of culture. Temperature, pulse, and respiration were taken every 4 hours and so

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antipyretics were used except for temperatures greater than 40° C. Daily observation was made for clinical symptomatology. The second method involved a 6 day protocol. The same methods were used except for the fact that on day 6 approximately 5 grams of tissue was removed for virus isolation and the study period terminated.

1. Methods of virus isolation: Swabs of vaginal discharge were immersed in 2 cc. of salt solution. This material was inoculated on Hela tissue cultures and observation made daily for cytopathogenic effect of Coxsackie type. Cervical biopsies were homogenized and this material was handled in a manner similar to the swab cultures. The 5 grams of tissue removed was sectioned. Half the material was used for Maitland culture and the other half homogenized for isolation as above. The harvested Maitland fluid was also inoculated on Hela cells.

2. Preparation of injectable material: Pools of injectable material were prepared by inoculation of a 32 oz. Hela bottle with the original material of one of the isolates. The harvested bottle fluid was used as a pool. The other source of injectable material was the filtered Maitland and homogenized fluid.

3. Safety tests: All material used for human injection received animal testing and tissue culture identification with specific antiserum prior to human use.

4. Antibody titers: Serum antibody titers to Coxsackie B5 virus were determined by neutralization in tissue culture using a 1:4 dilution of standard and unknown serum.

Part II.

Design of protocol:

1. Base line (5-7 days) - Clinical observations and routine studies. Diet = normal.

2. Control Period (12 days) - Blood studies, urine studies, weight records, intake and output records. Diet = B_6 deficiency supplemented with 5 mg. of B_6 daily.

3. Deficiency Period (variable) - Diet = B_6 deficiency + desoxypyridoxine.

4. Deficiency Period (with virus) - Diet = B_6 deficiency + desoxypyridoxine.

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5. Recovery Period I (variable) - Diet = B_6 deficiency + desoxypyridoxine + pyridoxine.

6. Recovery Period II (variable) - Diet = B_6 deficiency + pyridoxine.

7. Control - Normal diet.

Diet: 35 calories per kilogram weight.

Vitamins - Compound #102
Vitamin D - 800 u
Vitamin A - 5000 i.u.

Minerals - NaCl, 5 grams

Protein - 125 grams of vitamin free casein

Carbohydrate - Dextrose q.s. to make 2/3 of remaining calories

Fat - Corn oil q.s. to make 1/3 of remaining calories.

The diet is mixed in a Waring blendor with distilled water and distributed into 6 equal feedings which are taken over a 12 hour period. Distilled water is provided ad lib. Food flavoring is added to the diet. No other intake and no lotions or creams that might be vitamin containing are used.

Clinical observations: Daily clinical checks are made to ascertain any pertinent symptoms or physical findings that may be pertinent to the study. The patients were weighed daily and accurate intake and output records were kept.

Routine laboratory studies: A routine urinalysis, BUN, FBS, creatinine, uric acid, cholesterol, TSP A/G, Ca, P, Na, K, Cl, CO_2, alkaline phosphatase, BSP and Transaminase, Hgb, Hct, WBC with differential, RBC, and indices were performed at least once weekly by standard laboratory methods.

Special studies relating to B_6 deficiency: Whole blood transaminase studies were done twice weekly in one patient. Bone marrow aspiration was performed before and during deficiency in one patient. Xanthrenic acid excretion in the urine was quantitated after a load test of Tryptophane several times in each patient. This compound accumulates in B_6 deficiency. Other studies performed on some of the patients included creatinine clearance, PSP excretion, and Fishberg urine concentration tests. Electroencephalograms and

electrocardiograms were done when indicated.

Studies relating to tumor, virus, and host response to infection: Typhoid-paratyphoid vaccine, blood group substance, and Coxsackie B_3 virus were used as antigens and were given to the patient at a time determined by clinical and laboratory state. Serum was drawn 12-14 days later for antibody titer. Antibody against typhoid and paratyphoid was measured by slide agglutination, blood group substance by hemolysis of red cells, and virus antibody by neutralization in tissue culture. Virus cultures were done according to the previously described protocol for virus treatment of cancer. The cellular response of the host to an inflammatory stimulus is studied by the Rebuck technique which consisted of scraping skin down to the corium, applying a drop of Diptheria toxin, and covering the area with a slide. Differentials of cellular exudate are studied at 3, 6, 12, and 24 hours following inoculation. This procedure is performed before, during, and after the deficiency period. Tumor oncolysis is graded according to the previously described scale.

Patient Material:

Part I.

All patients used in this study were referred to the surgical branch with carcinoma of the cervix. In most instances this treatment was administered and followed by definitive surgical or radiation therapy.

Major Findings:

Part I.

1. Virus recovery (Table I): For the first four days following injection the attempt to recover virus was successful in almost all cases. Between days 4 and 8 the frequency of recovery dropped precipitously. Virus was recovered in only three patients after day 8 and in only one after day 11. Titers of virus in the vaginal discharge and biopsies revealed that coincident with a decline in frequency of virus recovery there was a decline in quantity of virus recovered. However, when all specimens from a patient are totaled, the quantity of virus recovered was greater than that administered. Recovery of virus from six immune patients was accomplished for 5 days following injection in one patient and for two days in another. In four others no virus was recovered.

Swab cultures of throat and anus were intermittently positive. No throat culture was positive after day 6 and no anus culture after

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day eight. Blood cultures taken routinely at two day intervals on two patients yielded only one positive, this on day two. At this time the patient was afebrile and asymptomatic and she remained so. All blood cultures taken at the time of fever or symptoms were negative. Tissues removed at postmortem examination in three patients were all negative for virus; however, this was many days after virus injection.

TABLE I

Virus Recovery from Cervix Cancer of 9 Nonimmune Patients

	Days Post Injection															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. Specimens	5	8	5	9	8	7	9	6	8	6	3	7	3	6	3	3
No. Positive	5	7	4	8	6	4	4	2	2	2	2	1	0	1	0	0
% Positive	100	88	80	89	75	57	44	33	25	33	67	14	0	17	0	0

2. Antibody formation (Table II): All patients demonstrated a rise in antibody titer following virus injection. The rise in titer began as early as four days following injection and by day 8 all patients had a titer greater than 4. The very early rise seen in some patients suggests the anamnestic response, which would indicate previous immunity had been present at some time. Patients with a preinjection antibody titer of greater than 4 demonstrated a rapid rise in titer. Absolute titers 10 to 14 days post injection varied between 256 and 2048.

3. Clinical results: Findings suggestive of systemic or localized virus infection were conspicuous by their absence. Mild fever occurred in 12 of 25 patients receiving Coxsackie B3 alone. This occurred from 12 to 72 hours after injection. Fever greater than 39° C. occurred in two patients. Leukopenia and lymphocytosis occurred in 5 of 17 patients followed closely. When both adenovirus and Coxsackie were given in combination to five patients, within twelve hours temperature would rise to 39-40° C. and undergo slow decline. Associated with this febrile reaction would be complaints of loss of appetite, weakness, headache, and generalized aching of muscles and joints, but absence of any physical findings. All blood cultures from these patients were negative for virus. The explanation and significance of this observation is unknown.

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TABLE II

Antibody Response in Patients Following Coxsackie B_3 Intra-tumor

	Days Post Injection							
	1	2	3	4	5	6	7	8
% Patients with Antibody >4	0	0	0	20	33	67	75	100

TABLE III

Tumor Oncolysis Resulting from Coxsackie B_3 Injection of Patients with Carcinoma of the Cervix

Category		No. Pts.	Oncolysis				
			0	1+	2+	3+	4+
Nonimmune, Coxsackie alone		19	11	0	5	2	1
Nonimmune, Coxsackie + Adenovirus		4	0	1	3	0	0
Subtotal		23	11	1	8	2	1
Immune	Coxsackie - 6 Combination - 1	7	4	1	2	0	0
Totals		30	15	2	10	2	1

4. Oncolysis:

Immune vs. nonimmune: (Table III)

Tumor oncolysis is graded on a scale of 0 to 4+. Of 19 nonimmune patients receiving Coxsackie B_3, 11 showed no oncolysis, 5 a 2+ response, 2 a 3+ response, and 1 a 4+ response. Use of the combination in nonimmune patients resulted in a response in all four with three of these being 2+. Of seven immune patients, two showed a 2+ response, one a 1+ response, and the other four no response. A significant response in immune patients was twice as frequent as that of nonimmune patients.

Cervical biopsies of the injected tumor have thus far failed to demonstrate specific histological changes that were shown in the animal system to be caused by virus.

Effect of Passage: (Table IV)

Two techniques of passage were used. The first consisted of using Hela cells for isolation and boosting virus titer between injections. The second system was using extracted and Maitland culture material from tumor removed 5 to 6 days post injection without intermediate tissue culture. Thus far this technique has resulted in insufficient quantities of recovered virus for maintaining passage. All passage has been accomplished by the first described method. Material through nine passages has been studied. The occurrence of 3+ and 4+ responses in late passage suggests that change may be taking place. If the four cases of third and fourth passage are removed, all four having also received adenovirus, the suggestion is enforced. The response frequency of the original virus and two passages is one in five or 20%, while that of the last five passages is 7 in 14 or 50%. If the two patients receiving pyridoxine deficient diet are removed, the response frequency is 42% in the last 5 passages.

5. Correlations with degree of oncolysis: The clinical stage of the tumor and the presence or absence of previous irradiation bore no relationship to degree of oncolysis. Likewise, the quantity of liquid and quantity of virus injected had no relationship to result obtained. The presence of signs and symptoms of systemic virus effect, namely fever, pain, and leucocyte changes, was not associated with more or less oncolysis than those patients without any systemic effect. There was a greater frequency of response, 50% as opposed to 36%, in patients over 50 years of age. Also the 3+ and 4+ responses were in patients over 50. Increased frequency and quantity of virus recovered was usually, but not invariably, associated with increased oncolysis. The most important

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TABLE IV

Effect of Passage of Virus on Oncolysis Produced in Nonimmune Patients

No. Pass.	No. Patients	Oncolysis					Response Frequency
		0	1+	2+	3+	4+	
0	2	1	0	1	0	0	1 in 2
1	1	1	0	0	0	0	0 in 1
2	2	2	0	0	0	0	0 in 2
3	2	0	0	2	0	0	2 in 2
4	2	0	1	1	0	0	2 in 2
5	3	2	0	0	1	0	1 in 3
6	3	1	0	1	0	1*	2 in 3
7	4	2	0	1	1*	0	2 in 4
8	2	2	0	0	0	0	0 in 2
9	2	0	0	2	0	0	2 in 2
Totals	23	11	1	8	2	1	12 in 23

* B_6 def. in conjunction with virus

correlation seems to be the absence of pre-existing antibody. When antibody was present, there was usually little or no response.

Part II.

This study is incomplete at present. There has been tremendous variability from patient to patient that summary conclusions are difficult. Consequently, the results will consist of case reports with certain generalizations and conclusions that seem valid at the present time.

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Case #1 (A.R.): This was an 80 year old colored female with extensive carcinoma in the pelvis. Pyridoxine deficient diet was instituted in conjunction with 200 mg. of desoxypyridoxine daily. Within one week nausea and vomiting appeared, and more confusion was noted (the patient was confused prior to the study). During the second week there was an increase in these symptoms and by the third week the patient became semicomatose. At this time the blood urea nitrogen was noted to be 83 mg.% and the serum creatinine 1.9 mg.%; both studies were previously normal. Renal function studies were performed which revealed a markedly decreased creatinine clearance and phenolsulfonphthalein excretion. During the last four days on the diet the patient lost 3½ kilograms or 2 lbs. per day while receiving a more than adequate quantity of calories and protein. After 19 days the deficiency diet was stopped and pyridoxine given. Within 24 hours the patient became responsive and nausea and vomiting ceased. By 48 hours the patient was sitting in a chair. By 6 days she was alert, active, ambulant, and had a good appetite. By 15 days the blood urea nitrogen and creatinine were normal. Repeat renal function tests showed increase in PSP output and creatinine clearance, though still less than predict. Between 15 and 20 days post deficiency, a superficial generalized desquamation of the skin occurred.

Case #2 (J.J.): This was a 68 year old colored female with stage IV undifferentiated carcinoma of the cervix. Pulmonary metastases were present. Pyridoxine deficient diet in conjunction with 200 mg. of desoxypyridoxine daily was started. Some mild lethargy was present on day 5. On day 13 the patient was noted to be emotionally labile and marked nausea was present. On day 20 scaling and fissuring of the angles of the mouth and lips occurred. On day 24 the patient was noted to be markedly confused. For the following 18 days she was noted to have periods of somnolence, periods of marked agitation and disorientation, and periods of quiet orientation. Nausea and vomiting was frequent. Skin changes progressed to increased pigmentation generally, and a dry, hard, leather-like skin developed. There was papillary atrophy of the tongue and marked rhinorrhea. On day 29 she was given antigenic challenge. On day four post virus, a superficial necrosis of tumor was noted. On day 8 ulcerated areas of tumor necrosis were present and the tumor was noted to be like jelly. By day 10 marked tumor necrosis was present and on day 13, at the time the deficiency was stopped, tumor necrosis was greater than 4+. Virus was recovered in greater than usual quantity through day 11. Antibody to virus during this time rose from < 4 to 128, for blood group substance from 64 to 256, and for typhoid from 1:20 to 1:80. There was no rise in titer to paratyphoid A. The patient tested by Rebuck technique just prior to stopping deficiency had essentially no late or mononuclear cell response to the inflammatory stimulus.

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Circulating white blood cells had risen from 13,000 mm.^{3} to 22,000 mm.^{3} During the last 10 days of the deficiency the patient lost 10 kilograms in weight or 2 lbs. per day in the presence of adequate protein and calorie intake. Associated with this was a rise in blood urea nitrogen from a normal level to 147 mg.%. The serum creatinine rose from 0.9 mg.% to 2.7 mg.%, and the FBS from 110 mg.% to 220 mg.%. There was good urinary output at the time. Creatinine clearance and PSP excretion tests revealed marked impairment of renal function. Xanthrenic acid excretion after 5 grams of tryptophane rose from a control value of 19 mg.% per 24 hours to 1973 mg.% per 24 hours on day 28. On day 42 deficiency diet was stopped and pyridoxine instituted. Within 24 hours nausea and vomiting had ceased and the patient was oriented. Within 48 hours there was apparent fissure healing and scaling of thick layers of skin generally. From 48 to 72 hours the patient complained of "burning of the fingertips." She remained oriented and skin continued to scale. It was 6 weeks post deficiency before all evidence of scaling had ceased. No weight loss occurred after the addition of B_6, and by 20 days post deficiency BUN, creatinine, and FBS were normal. Repeat renal function tests were improved, but had not returned to previous levels. All evidence of pyridoxine deficiency disappeared. However, for 4 weeks following cessation of the deficiency, tumor necrosis and slough continued. The patient never fully regained strength and a terminal 2-3 day coma followed by death occurred. Postmortem was obtained which revealed the cause of death to be necrotizing renal papillitis. No histological evidence of pyridoxine deficiency was found. A 3-6 mm. rim of viable tumor remained in the uterus. The pulmonary metastases had necrotic centers and numerous viable pulmonary metastases were also found. The cell type at postmortem most resembled sarcoma.

Case #3 (E.J.): This was a 57 year old colored female with stage IV carcinoma of the cervix with vesicovaginal fistula and bilateral hydro-ureter and hydronephrosis. Diet was started in association with 200 mg. of desoxypyridoxine daily. No change in the patient's status was noted for 16 days except more frequent temperature spikes which had been present pre diet. Days 14 to 20 were characterized by slow progression to a semicomatose state, persistence of high fever and CVA tenderness in the presence of antibiotics. A blood culture on day 20 was positive for proteus and the patient unresponsive to additional antibiotic therapy. Blood urea nitrogen was 25, and monocuclear response at 24 hours was 40%. The patient died of septicemia without evidence of pyridoxine deficiency.

Case #4 (B.B.): This was a 57 year old colored female with stage IV inoperable carcinoma of the cervix. Diet was instituted in conjunction with 50 mg. of desoxypyridoxine daily. On day 1 the

patient was noted to be drowsy and somnolent and requiring less pain medication. An occasional temperature spike occured. On day 12 emotional lability was noted with mood changes rapidly from euphoria to depression and easy crying. On day 13 the BUN was noted to be 43 with a creatinine of 1.3. Protein in the diet was decreased to 50 grams per day. The BUN returned to normal and remained there; however, the serum creatinine rose to 1.8. On day 20 vomiting appeared for the first time and on day 21 for a period of 6 hours the patient's blood pressure was 220/120 associated with headache and dizziness. Emotional lability continued, but for the majority of the time the patient was oriented. At 14 days the xanthrenic acid excretion was 484 mg. per 24 hours. On days 21 and 22 antigen challenges were given. Virus recovery was successful through day 10, and 3+ tumor oncolysis occurred. Antibody to Comsackie B_3 virus rose from < 4 to 64. Blood group substance rose from 64 to 128. Vomiting was present for the last 5 days and during the last 2 days some dry scaling of the cheeks was noted. There was no weight loss. Other pertinent laboratory studies were a decrease in PSP excretion, creatinine clearance, and serum and whole blood transaminase. From days 34 to 41, 300 mg. of pyridoxine per day was given in addition to B_6 deficient diet and desoxypyridoxine. Within 24 hours she was oriented, stable, and all nausea and vomiting had ceased. Skin changes were gone in 3-4 days. Desoxypyridoxine was discontinued for days 41-48 with no other change in diet. There was no change in the patient. After being given normal diet no change was noted. All abnormal laboratory studies reverted to normal with only the addition of pyridoxine.

Summary:

1. Resistance to infection: Of the three modalities of resistance to infection studied, namely antibody formation, cellular migration to an inflammatory stimulus, and peripheral leucocyte counts and differentials, only one has shown a suggestion of being influenced by B_6 deficiency. This modality is cellular migration to an inflammatory stimulus. The 24 hour mononuclear response, particularly with high doses of desoxypyridoxine, is markedly impaired. There is a suggestion that antibody formation is delayed and absolute titers low. More cases may statistically prove this to be so. Virus seems to survive longer in the tumor than usual and degree of oncolysis in two cases was greater than that in 28 cases without associated B_6 deficiency.

2. Previously reported findings in human pyridoxine deficiency: The mental symptoms are different in that mood swings with periods of stability and orientation are more frequent than those previously reported. The skin changes in J.J. were far more severe than any reported. Only two patients exhibited a marked lymphopenia. Other

blood changes could not be studied in these patients as all were bleeding during study. The markedly elevated xanthrenic acid excretion in patient J.J. was much more than that reported by other authors. It is felt by this observer that the changes seen were consistent with those previously reported, but in certain instances were more severe than any reported.

3. Unreported findings: The markedly impaired renal function and the profound catabolic responses are heretofore unreported. The explanation and significance of these findings is unknown at present. The elevated fasting blood sugars are also unreported, but may be explained by the known toxicity of xanthrenic acid for the B cells of the islets of Langerhans.

Significance to Cancer Research:

Part I.

Exploration of new avenues of treatment of cancer in the human is a necessity. New information is being gained on the nature of virus infection in the human.

Part II.

This study demonstrates a means by which a treatment of human cancer can be enhanced. Studies on mechanisms of host immunity may aid in the understanding of human immunity to cancer. The expansion of knowledge on human physiology and biochemistry will aid in the understanding of many neoplastic and other diseases which affect man.

Proposed Course of Project:

Part I.

In order to further the encouraging effects obtained thus far in this study, it seems imperative that we know if the virus enters the cell and cell death results. There are techniques available that can provide this answer. If this is true, and all indirect evidence suggests that it is, then one must know what the limitation is on degree of oncolysis. If it is a matter of virus contact with all cells, then all possible routes of infection must be used simultaneously. If it is host resistance to infection, then this must be altered sufficiently to produce the desired oncolysis without detriment to the host. These are the planned approaches and it is felt that this can be done in the virus passage situation. Ultimately, the specific biochemical upset of the cell as a result of virus infection will be sought.

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Part II.

Two more human cases at lower desoxypyridoxine dosage are planned. Further study on the renal function and catabolic abnormalities are planned on laboratory animals.

Part I included: Yes [ ] No [X]

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1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: I. Determination of the Frequency and Significance of the Finding of Tumor Cells in Washings Obtained from Operative Wounds Following Definitive Surgery for Cancer.

II. Determination of the Frequency and Significance of the Finding of Tumor Cells in Blood Obtained from Peripheral Vein or Veins Draining a Cancer in Patients Undergoing Primary Cancer Therapy.

III. Adjunctive Chemotherapy of Cancer Operative Wounds.

Principal Investigators: Dr. Robert R. Smith and Dr. John F. Potter

Other Investigators: Dr. Richard A. Malmgren, Dr. George H. Longenbaugh, Dr. John S. Dillon, and Dr. Marvin M. Romsdahl

Cooperating Units: Cytodiagnostic Service, Pathologic Anatomy Branch, NCI, and the USPHS Hospital, Tumor Service, Baltimore, Maryland

Man Years (calendar year 1959):
Total: 6½
Professional: 2
Other: 4½

Patient Days (calendar year 1959):

Project Description:

Objectives:

To determine the frequency and significance of finding tumor cells in wound washings at the time of definitive cancer surgery and the development of methods of prevention of wound implants.

To note the frequency of detection of cancer cells in the peripheral venous system and in the local venous system draining the tumor in specific types of cancer. To note the effect of stage of disease on the incidence of the recovery of tumor cells. To observe the correlation between specific pathologic analysis of the surgically removed specimen and the recovery of tumor cells

in the peripheral and local venous blood. To note the effect on prognosis of the presence of tumor cells in peripheral and local venous blood. To correlate the detection of tumor cells in the peripheral venous blood of patients with malignant melanoma with the development of metastatic skin tumors on a quantitative basis. To note the effect of hormonal ablative surgery on the incidence of circulating cancer cells in patients with cancer of the breast.

Methods Employed:

Part I. Patients with primary operable cancers of the head and neck, pelvis, or other appropriate sites, with regional lymph node metastases, or suspected metastases, have been admitted for primary surgical therapy. For the most part, these cases have exhibited metastases. The surgery performed is the standard operative procedure accepted as treatment of choice for the specific cancer site.

Following removal of the tumor and its metastases, the operative area is thoroughly washed with a fine saline spray. The aspirate is then prepared for cytologic study.

Part II. (Dr. Potter - Tumor Cells in Blood) An extensive study has been embarked upon to elicit basic biologic information on the dissemination of cancer through the blood stream. It is expected that this information may be obtained by the detection of cancer cells in the blood stream of patients.

The isolation of cancer cells circulating in the blood is performed by a technique previously reported. Briefly, detection of these cells is accomplished by incubating blood cells after the removal of plasma with Streptolysin O. This accomplishes hemolysis of the red cells and polymorphonuclear leukocytes. The resultant suspension of tumor cells and lymphocytes is filtered through a Millipore filter and is stained with the Papanicolaou technique. Cytologic screening is then performed.

Clinical studies are underway on patients at the National Cancer Institute, Bethesda, and on patients from the Tumor Service of the USPHS Hospital, Baltimore. On admission to either of these institutions, blood specimens are drawn. If this patient comes to operation, multiple blood specimens are obtained in the operating room. Specimens are obtained from the peripheral venous circulation, from the local vein draining the tumor, and from a systemic artery, both before and after operative manipulation of the tumor. In addition, wound washings are taken of these patients for cytologic examination. After surgery or other courses of

treatment, periodic examination of the blood for tumor cells is made. Avenues of research that are currently under investigation include:

1. Correlation of the presence of cancer cells in the blood with specific types of tumor. This is being accomplished by drawing blood specimens on any patient with cancer and comparing the percentage of positive findings in different tumors.

2. Correlation of tumor cell counts in the blood with the development of countable metastases. This study is being undertaken to determine the nature of the response involved in the development of metastases.

3. Correlation of the blood findings obtained during operation and the wound washings with the development of local recurrence or distant metastases. This is a long-term project which will require several years for suitable analysis.

4. To note any effect in prognosis afforded by the detection of circulating cancer cells.

Part III. After removal of the cancer and before closure of the operative wound, saline washings are taken. This is followed by a wound wash of ±200 cc. of 0.5% formaldehyde. The wound is then closed in a routine manner. In addition, during the past year suture material treated with picric acid has been used in selected cases in addition to the formaldehyde wound wash in order to prevent local recurrent cancer.

Patient Material:

Patient material for this project was supplied by patients with primary operable but advanced cancers of various sites. Experience with the study was also obtained by necessary surgery on patients admitted for other projects. To date, approximately 300 operative procedures, with wound washings, have been performed.

Patient material for blood studies has been derived from those patients hospitalized or followed as outpatients at the National Cancer Institute and the Public Health Service Hospital in Baltimore, Maryland. In the year since the previous annual report 794 specimens have been processed, screened, and definitely diagnosed from the Bethesda activity. A total of 809 specimens have been processed at Baltimore in this period. These slides are currently being screened and it is expected that screening will be up to date in four months.

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Major Findings:

Part I. (Wound Washings) During the past year wound washings from head and neck cancer cases have been reviewed. 107 operative procedures were done: 88 for epidermoid carcinoma and 19 for non-epidermoid cancer. In the 88 epidermoid cancer wounds, positive washings were observed in 26% (the same value previously reported for larger group of operations). It was noted that positive washings were most frequently encountered when a radical resection of primary tumor only was performed. When a radical neck dissection alone was done without combining it with resection of the primary tumor, only one suspicious washing was reported and no positive smears were seen. Positive washings were more frequently seen when surgical margins were not clear of tumor. For example, in the negative washing group of cases, 94% had surgical margins clear of tumor, the suspicious group 180%, while the positive group had only 61% of the cases with clear surgical margins. Local recurrance could not be correlated with wound washing findings. During the period of study, 33% of the negative group and 38% of the positive group developed local recurrent cancer.

Part II. The data obtained from the above outlined investigations is currently being tabulated and reviewed. In a group of 285 patients, 79 or 28% had malignant cells in an antecubital vein. If this same group of patients is categorized into "resectable" and "non-resectable" cases in an effort to correlate the detection of malignant cells with progression of disease, it is noted that the recovery rate was 16% (19 of 117 patients) in the resectable group and was 36% (60 of 168 patients) in the non-resectable group.

Blood specimens obtained from a vein draining the tumor site have been analysed in 91 patients. Thirty-nine (43%) of these patients have been positive. Of the 75 resectable patients, 30 (40%) had positive cells at the time of operation in the local venous blood. There were 16 patients who were classified as non-resectable. Of these, 9 (56%) had positive local blood.

If the results are analyzed by specimen instead of by patient, the percentages of positivity decrease due to the decreased size of the sample in the single specimen. Thus, in the peripheral blood, the percentage of positive specimens in the resectable group is 9%, and is 15% in the non-resectable group.

In the local blood category, 30% of the specimens are positive in the resectable patients and 48% are positive in the non-resectable group.

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The recovery of tumor cells from specific tumors has been analyzed. The results here are open to the criticism that the proportion of resectable to non-resectable patients in each tumor group is not constant. Further sampling will eliminate this disadvantage. At present, in the peripheral blood 47% of the melanoma patients are positive. Cancer of the breast was 39% and choriocarcinoma was 34%. Sarcoma patients were 29% positive and cancers of the genitourinary tract and the cervix each showed a 23% incidence.

In the local blood, 66% of sarcoma patients and 64% of cervix cancer patients were positive. Epidermoid cancer of the head and neck was positive in 35% of patients and gastrointestinal neoplasms were positive in 26% of patients.

The positive blood specimens have been analyzed on a quantitative basis. Positive peripheral blood specimens totaled 1284 cc. In this volume there were 1161 single cells (0.9 cell/cc.) and 67 groups of cells (.05 group/cc.). A group is arbitrarily defined as a cluster of more than five single cells.

Positive local blood specimens totaled 377 cc. In this volume were 2062 single cells and 193 groups of cells. The frequency was thus 5.5 cells/cc. and 0.5 group/cc.

In addition to these statistical findings, qualitative observations have been possible. These include the observation that apparently early clinical cancer can produce neoplastic cell emboli. In following a postoperative patient after a definitive surgical procedure the recovery of malignant cells is low, but when present, has a poor prognostic significance.

Studies of blood from patients with malignant melanoma show:

1. Circulating tumor cells have been recovered from peripheral venous blood in approximately 75% of the cases and in 18% of all specimens obtained from the group with advanced disease and receiving chemotherapy.

2. Six patients have been studied who had surgically resectable disease. Positive samples were obtained from two patients preoperatively, from two patients during surgery from regional blood draining the tumor area, and from three patients postoperatively. The two patients with positive blood samples from regional veins both have developed recurrent disease and expired. These two patients, also, are included in the three patients with positive postoperative specimens.

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Sixteen choriocarcinoma patients have had circulating tumor cell blood studied by Dr. Dillon. Initial blood studies showed tumor cells in five instances. Of a total of 118 blood specimens in the 16 patients, 11.9% were positive and 13.6% were suspicious. Eight of the 16 patients had at least one positive specimen some time during the period of study (50%). Four additional patients had only a suspicious specimen report and the remaining four never had a positive or suspicious blood. A positive specimen was never found when the urinary gonadotrophin titer was normal and the chest x-ray revealed no metastases. Attempts to relate positive blood specimens to prognosis, treatment, and the presence of chest metastases failed. Positive peripheral blood specimens are believed indicative of residual or metastatic disease, but the results in general do not correspond closely to clinical status.

It is interesting to speculate regarding the relatively small number of positive specimens in choriocarcinoma. This disease, like melanoma, is known to spread by hematogenous means, and one would expect a higher incidence of positive blood findings than in a disease like epidermoid cervix cancer.

Twenty-nine patients with carcinoma of the breast have been studied. A total of 149 blood specimens have been drawn and processed. 27 of the 149 specimens have been positive (18.8%) 34 have been suspicious (22.8%), and the remainder (58.4%) of the specimens have been negative. Only 4 of these patients had circulating tumor cells in the initial specimen. 13 of the 29 had positive specimens at some time during the study, 9 more patients had suspicious specimens but no positive specimens, and 7 of the 29 were never found to have either positive or suspicious specimens.

A small number of patients with prostatic carcinoma has been followed. 6 patients in this study had a total of 18 specimens drawn. 3 of these patients have had only one specimen, and a fourth has had only two. The data with respect to the patients then is probably not significant. The 18 specimens, however, yielded 4 positive specimens (22.2%) and 7 suspicious specimens (38.9%).

Part III. (Adjunctive Chemotherapy of Operative Wounds) During the year a statistical analysis of followup data on a group of cervix cancer and head and neck cancer patients was completed. These patients had received 0.5% formaldehyde wound wash after completion of standard operative procedures. Local recurrence experience is given in the following table:

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Cervix Group	Total Proportion of Local Recurrences	Cumulative Proportion of Local Recurrences Adjusted for Followup	
		To 6 mo.	To 12 mo.
Formaldehyde Treated	3 in 22 = 14%	.06	.20
Untreated	10 in 23 = 43%	.18	.27
Head and Neck Group			
Formaldehyde Treated	11 in 31 = 35%	.38	.38
Untreated	12 in 33 = 36%	.16	.27

Life table analysis of survival and local recurrence experience in the cervix and head and neck cancer groups revealed no significant difference when compared to similar untreated groups of patients. This disappointing ineffectiveness of local formaldehyde treatment can possibly be explained on the basis of lack of contact of the drug with the cancer cell implants. Transplantation of clumps of cells by suture thread has been demonstrated. Possibly we have been transplanting the implants with suture material. At the present time a series of patients are under study in which the formaldehyde spray has been supplemented by use of picric acid treated sutures. However, as additional experience is obtained, the hope of an effective local chemotherapeutic measure becomes less tenable. Do operative wounds become reseeded from cells in circulation? A better understanding of the many factors associated with metastases and implantability of loose tumor cells will help answer this problem.

Significance to Cancer Research:

This study is designed to elicit information on cancer biology and aid in the evaluation of adjunctive chemotherapy.

It is believed that long term clinicopathologic studies of this type will add significant data to the study of metastases.

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At the present time, only preliminary and possibly the least informative data has been collected. Further analysis of the cases already screened and cytologically diagnosed should be informative.

This project is significant to cancer research inasmuch as it provides physiological information relative to the phenomena of metastases. If the correlations outlined can be established, the examination of the peripheral and/or local regional blood may be used to provide information relative to prognosis and perhaps to other physiological factors involved in host resistance.

Proposed Course of Project:

During the coming year we hope to continue wound washing cytology studies and studies on local and peripheral blood of patients obtained at the time of surgery. A more detailed study of the material that has been obtained will be made. The effect of circulating tumor cells in specific types and stages of cancer needs to be analyzed in order to determine their influence on prognosis of the individual cancers.

A more extensive study of early malignant melanoma patients, correlating tumor cell counts with the development of metastatic nodules, should be quite revealing. At present, our efforts in this regard have been hampered by the lack of suitable clinical material. However, a satisfactory clinical model has been developed, and with access to earlier clinical stages of melanoma, valuable information on tumor cell dose response curve should be obtainable.

Followup studies of patients who have had definitive surgery will be undertaken as part of the program to evaluate the prognostic significance of cancer cells in the local and peripheral venous system.

In addition, there are certain areas for detailed study which might prove fruitful. Very close observation of a single patient, with multiple sampling through a 24 hour period and for a period of several days, might clarify a possible variation in the dissemination of cancer cells. This study would probably be facilitated by the use of intravascular polyethylene catheters.

Further studies should also be undertaken to note the influence of capillary network or networks on the tumor cell count. This would be achieved by study simultaneously of specimens from the vein draining the tumor (for example, melanoma of an extremity), arterial blood (to obtain a specimen which had crossed

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one capillary network--the lung), and from the peripheral venous system (to obtain a specimen which had traversed two capillary networks).

Part B included: Yes [XX] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, Robert R. and Gehan, Edmund A.: The Effect of Formaldehyde Wound Wash on the Development of Local Wound Recurrences. J.N.C.I. 23:6, 1339-1345, 1959.

Potter, John F. and Malmgren, Richard A.: A New Technique for the Detection of Tumor Cells in the Blood Stream and Its Application to the Study of the Dissemination of Cancer. Surgical Forum. Vol. IX, 580-583, 1959.

Honors and Awards relating to this project: None

Serial No. NCI-8-754
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
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Part A.

Project Title: Evaluation of the Nature, Cause and Means of Preventing Seeding of Operative Wounds Using Transplantable Animal Tumors.

Principal Investigator: Dr. Alfred S. Ketcham

Other Investigators: Dr. Seymour C. Nash and Dr. Chester L. Haverback

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2½
Professional: 1
Other: 1½

Patient Days (calendar year 1959):

Project Description:

Objectives:

To study local seeding of operative wounds and to develop methods of its prevention, using laboratory animals as a model. To show that tumor cells can be transplanted in a wound by means of suture material, and development of a suitable chemical agent that can prevent this means of dissemination.

Methods Employed: (Dr. Ketcham)

In previous studies reported under this project, it has been demonstrated that chemotherapy of cancer, as applied by the surgeon, requires an agent to be effective on implants not yet growing in its metastatic site. This led to the development of artificially seeded operative wounds and the subcutaneous implantation of tumor inoculum by the air bubble technique. These techniques were applied in an *in vitro* group of studies in which the inoculum, after being removed from the donor animal, was pre-treated with the drug to be used before placing the inoculum into the test animal. This gives direct information regarding any oncolytic effect the drug under study may have when placed in direct contact with tumor cells.

In vivo studies more nearly approximate conditions seen in the treatment of human cancer by surgical means. In these [illegible]

the tumor inoculum was placed in the operative wound, in an air bubble, intraperitoneally or intravenously. The drug to be studied was then applied at varying intervals of time. The effectiveness of the treatment can be measured by degree of inhibition of tumor growth. In all instances, toxicity studies were performed to determine the effect of the drug on normal tissues.

This report will deal with further studies of Clorpactin XCB and additionally with the evaluation of another commercial hypochlorous acid derivitive, Clorox, as well as a preliminary evaluation of alcohol, hydrogen peroxide and ThioTEPA.

Hypochlorous Acid Study - The bulk of the work has been done with two animal tumors, S-91 melanoma with CDBA mice and K-2 ascites tumor with NIH white mice. In all tumors evaluated and discussed in this report, the inoculum is prepared in a like manner. One gram of viable tumor tissue is prepared by the Small cytosieve technique to a 1:10 dilution, and then this inoculum placed either in the subcutaneous pouch raised on the back of the mouse by first injecting air or into an open wound brought about by blunt dissection. Groups of animals were first pre-treated with the drug to be evaluated, following which tumor was placed into the wound. This pre-treatment evaluation was carried out by placing the drug either in the pocket into which the drug was to be placed, or intraperitoneally to test its effect upon the subcutaneous tumor inoculum. In vitro mixtures of appropriate amounts of drug and tumor were similarly placed into wounds to determine their in vitro tumoricidal activity. The in vivo experiments, as with the pre-treatment experiments, were carried out at listed time intervals between the tumor inoculum and the drug. Throughout the closed wound studies, 0.1 cc. of tumor suspension and 0.5 cc. of drug were used. In the open wound experiments, 0.2 cc. of tumor suspension was used and 25 to 50 cc. of drug solution. Repeated control studies were run with all groups.

The open wound technique was developed in an attempt to more adequately simulate the operating table. The axilla and/or the groins were dissected to expose the nerve and vascular plexus and following the appropriate therapy the wound was closed with skin clips.

All animals in the S-91 studies were sacrificed on the [illegible] day and a visual estimate of the amount of tumor recorded by comparing with one another and using a scale range of 0 to 300.

All of the K-2 tumor injected animals were followed daily until the tumor first became palpable and that day recorded as

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the day of tumor presentation. These mice were all followed until death and that day recorded.

Methods Employed: (Dr. Nash)

Use of Proflavine hemisulfate and 9 amino-acridine - Left axillary incisions simulating radical mastectomy wounds are made in anesthetized DBA Pauley grey mice seven to thirteen weeks of age. These incisions provide a pouch into which .05 cc. of a tumor suspension of S-91 Cloudman melanoma is placed. The suspension is made aseptically using one gram of tumor taken from a thigh implant of another DBA mouse carrying S-91 melanoma. This is diluted with 10 cc. of isotonic sterile saline and passed through the Snell cytosieve. Approximately 20 to 30 minutes after inoculation, control axilla are then sprayed with isotonic saline for one minute, delivering approximately 14½ cc. of fluid. The wounds are then closed with 5-0 silk. Treated axilla are sprayed with various concentrations of Proflavine hemisulfate and 9 amino-acridine for one minute. Incisions are then closed with 5-0 silk. Four dilutions of the acridines are used, varying from 1 to 5,000, 1 to 1,000, 1 to 500, and 1 to 100. The solutions are made by dissolving a measured amount of drug powder with isotonic sterile saline. Each solution is made approximately one hour prior to use and kept in brown stoppered bottles. The above system is also used with inoculation of mast cell tumor suspension made as the S-91 melanoma suspension. The experiment is divided into four groups corresponding with the different strengths of drugs used. Each group contained treated and control animals inoculated from the same tumor. Animals are observed for tumor development.

Major Findings: (Dr. Ketcham)

The preliminary Clorpactin XCB studies presented in a previous report are listed below:

1. Pre-treating the wound with Clorpactin before tumor is injected enhances tumor growth.

2. Treating a wound contaminated with tumor with Clorpactin solution decreases tumor growth at concentrations above the recommended 0.5%. The higher concentration is more adequate in having a tumoricidal effect, but the drug becomes toxic at the 2 to 4% level.

3. The *in vitro* test tube mixture of tumor and Clorpactin completely depresses all tumor growth.

4. Human serum inactivated Clorpactin except at a high [illegible] concentration.

5. Increasing the volume of Clorpactin in the closed wound did not significantly depress tumor growth and at the 2 and 4% concentrations was toxic to the animals.

The accompanying table is a brief summary of an extensive survey using K-2 ascites tumor and varying concentrations of Clorpactin and Clorox.

TABLE I

Days It Took K-2 Ascites Tumor to Develop in Subcutaneous Pouch -- 1,000 Animals

Drug	Pre-treatment			In Vivo			In Vitro	Control
	24 hr	1 hr	30 min	1 hr	30 min	5 min	15 min	
Clorpactin 4%	9	9	8			11*	N	
Clorpactin 2%	14	8	8	13	10	15	O	
Clorpactin 1%	10	10	11	13	14	15	C	
Clorox .06 cc.	11	8	10	13	13	16**	R	12
Clorox .03 cc.		9	9	14	16	13	O	
Clorox .015 cc.		10	9	13	17	16	W T	
Clorox .0075 cc.		12	10	11	12	16	N	

* 4% is toxic -- ½ animals died, ¼ developed no tumor, ¼ had tumor on 11th day
** 20 of 60 animals developed no tumor

Results:
1. Pre-treatment accentuation in nearly all groups.
2. In vivo results not encouraging. 4 to 5 days of delay in tumor growth was the best seen. At .06 cc. Clorox at 5 minutes there was a 4 day delay, but additionally 1/3 of the animals did not develop tumor.

Again there was found a pre-treatment accentuation of tumor growth. The only encouraging tumoricidal activity of either drug in the *in vivo* evaluation where tumor was followed by the drug was using a concentration of Clorox which had .06 cc. diluted to ½ cc. of water. There was a four day delay in tumor growth, but additionally 1/3 of the animals did not develop tumor. The *in vitro* mixture resulted in no tumor growth.

Using S-91 tumor, Clorox again showed at both a .06 cc. and a .03 cc. concentration some depression of the amount of tumor growth seen as compared to the controls, but not of a statistically significant degree.

As a matter of interest, it should be mentioned that Clorpactin XCB has an active OCl content of 7.25%. Clorox is a 5% solution of NaOCl. It can be calculated that 0.6 cc. of Clorox equals 2 mg. of OCl. 0.5 cc. of a 1% Clorpactin solution equals .35 mg. of OCl. Therefore, the Clorox has a much greater content of the active ingredient OCl in equal amounts of Clorpactin 1%. It is theorized that the OCl is liberated more slowly in the Clorpactin solution and thus the reason for the lower concentration of OCl being affected.

Complete toxicity evaluation of these drugs is not being presented, but of interest is the open wound rabbit axilla toxicity studies. Clorpactin, 1 cc. in a 250 cc. volume, was compared to Clorox. In a similar volume of a .06 cc. concentration, both 1% and 2% Clorpactin solutions gave a partial weakness of the corresponding extremity, but by the 45-60th post-treatment day there had been recovery from this paralysis. The .06 cc. concentration of Clorox gave complete paralysis which did not recover, followed for over 90 days. A .015 concentration of Clorox gave paralysis from which the animal recovered similar to the Clorpactin concentrations.

Alcohol Studies - A 25% solution of alcohol, using 0.5 cc. in a subcutaneous pocket, was tolerated adequately by the animals. Higher concentrations brought about death.

In vitro mixtures of this 25% alcohol and tumor resulted in no growth of tumor when placed in the animal pouch. In the following table (Table IX) alcohol was used to treat S-91 and K-2 ascites tumor animals. There is again a pre-treatment augmentation at the listed time interval. The *in vivo* attempts to depress tumor growth were only moderately successful when comparison with the control was made. No growth was seen when the tumor was immediately followed by the alcohol solution using K-2 ascites. This was felt very likely to be an *in vitro* type of activity in this small subcutaneous pouch.

TABLE II

25% Alcohol in Subcutaneous Pouch

Time	S-91 (0-300) amount tumor growth	K-2 day tumor developed
Pre Rx		
5 min	225	6
1 hr	210	7
8 hr	206	7
24 hr	180	8
In Vivo		
immed	35	no growth
5 min	85	13
30 min	90	12
60 min	120	10
In Vitro		
10 min	no growth	no growth
Controls	125	10

Hydrogen Peroxide Studies - 0.25 cc. of a 1% solution of hydrogen peroxide was the highest concentration tolerated. Table XXI presents the results using K-2 ascites tumor, S-91 melanoma, T-241 sarcoma, and DBA-49. With all tumors there is complete *in vitro* tumoricidal activity and the previously noted pre-treatment accentuation of growth. *In vivo* activity with all tumors is present but not statistically significant when compared with the control animals.

TABLE XXI

Days It Took Tumor to Develop Using .25 cc. 1% H_2O_2

Time	K-2	S-91	T-241	DBA-49
Pre Rx				
5 min	6	11	9	6
30 min	7	13	10	6
60 min	8	16	12	8
In Vivo				
5 min	17	24	18	14
30 min	14	25	15	12
In Vitro				
15 min	no growth	no growth	no growth	no growth
Controls	13	20	15	10

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Merthiolate Studies - Using 0.5 cc. amounts, concentrations of merthiolate were toxic to the animal until the 0.2% solution was used. Listed in Table IV are the results of this drug and the appropriate tumors. Again, essentially identical findings are noted.

TABLE IV

Days It Took Tumor to Develop Using .5 cc. of 0.2% Merthiolate

Time	K-2	S-91	T-241	DMA-49
Pre Rx				
1 hr	10 4 died	16	10	9 4 died
In Vivo				
5 min	8 of 10 died	24	14	15 3 died
30 min	7 of 10 died	22	13	all dead
In Vitro				
15 min	no growth	no growth	no growth	no growth
Controls	12	20	12	10

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Serial No.

ThioTEPA Studies - Table V presents the studies using the listed concentration of this drug. The pre-treatment accentuation was consistent. Except for T-241, the other three tumors showed the most notable depression of their growth thus presented. There was a statistically significant depression of growth as listed by the increased number of days it took tumor to develop. This was most notable with S-91 melanoma.

TABLE V

Days it Took Tumor to Develop Using .5 cc. of 0.05% ThioTEPA

Time	K-2	S-91	T-241	DBA-49
Pre Rx				
1 hr	19	20	11 4 died	10
In Vivo				
5 min	20	55	10 4 died	20
30 min	19	39	11 4 died	18
In Vitro				
15 min	no growth	no growth	no growth	no growth
Control	13	24	10	11

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Summary - Pre-treatment accentuation has been noted with most drugs tested. The degree varies depending upon the amount of drug used and the time the drug precedes the tumor inoculation. This was presumed to be a mechanical irritating effect setting up an inflamed vascular area as an ideal site for increased tumor growth. However, in all the studies presented in the preceding tables where pre-treatment evaluation was done, there was a concurrent intraperitoneal pre-treatment study performed. A like amount of drug was placed intraperitoneally and then at the intervals listed in the tables, the inoculum placed subcutaneously. In nearly every instance there was pre-treatment accentuation of tumor growth of a degree which was less than that seen with the subcutaneous pre-treatment inoculation, but varying only by a day or two. This suggests that this phenomenon of pre-treatment accentuation is due to some unknown intrinsic systemic mechanism rather than a pure local mechanical effect.

Further Open Wound Evaluation with K-2 Ascites Tumor - Two methods were used to irrigate these open wounds. The first was by simple 50 cc. syringe forceful irrigation of the wound which had had tumor gently massaged into the wound and left for five minutes. The second was using 50 cc. of solution with a high pressure spray which followed the .2 cc. of tumor inoculation by five minutes. Both axillae, and in some instances, both groins were exposed, isolated from one another to prevent cross contamination, and used in the evaluation as to whether tumor would grow or not. By simple syringe irrigation, 50% of the animal wounds developed no tumor growth when saline, varying concentrations of Clorpectin, or Clorox was used. The exception to this was in the .03 cc. and the .015 cc. concentration of Clorox where 25-30% of the animals only developed tumor in their wound. Using the high pressure spray, again with varying concentrations of Clorox, Clorpectin, and adding to the protocol, 50 and 25% alcohol and .2% merthiolate and distilled water, the results with the drugs were essentially similar with a 50-60% instance of tumor growth to those washed only with saline. An exception to this was the 50% concentration of alcohol, where 30-35% of the wounds developed tumor.

The results of this rather extensive open wound protocol suggest strongly that there may be some increased tumorcidal activity to using one of the agents, particularly alcohol or Clorox, but the essential findings are similar to those found when only saline alone is used. Thus, the pure mechanical effect of washing a wound with saline is most advantageous as suggested in last year's annual report. It might be augmented somewhat by the additional use of a tumorcidal agent.

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Major Findings: (Dr. Nash)

Four pilot studies have been performed using four concentrations of proflavine hemisulfate: 1 to 5,000, 1 to 1,000, 1 to 500, and 1 to 100. These pilot studies have used a suspension of S-91 melanoma placed into a left axillary incision. These animals were divided into saline control sprayed groups and treated groups. The first group was treated with a dilution of 1 to 5,000 proflavine hemisulfate. Out of 14 saline spray control animals, 12 animals developed tumor in 30 days ranging from 46 mg. to 4.7 g. Two animals did not develop tumor or any gross evidence of melanin deposit in the incisions. In the treated group, 7 out of 18 animals did not develop nodules. However, there was pigment staining of the axilla. The remaining animals developed tumor varying from 56 mg. to 2.6 grams. This group was sacrificed at 30 days. In the second group, treated with 1 to 1,000 proflavine hemisulfate, the animals were sacrificed at 30 days. Ten saline control animals developed tumor ranging from 28 mg. to 4.2 g. In all ten treated animals there were no tumor nodules grossly. However, there was pigment staining of the axillary connective tissue. Pathology tissue sections were taken of several of these axillae, and on microscopic examination clumps of melanin containing tumor cells were seen. In the third study group three out of ten saline control animals did not develop any tumor and did not have any pigment staining of the axilla. The rest of the animals all developed tumor ranging from 10 mg. to 1.3 grams. In the treated group, with 1 to 500 dilution of proflavine, all animals failed to develop tumor nodules. However, pigment staining of the axillary connective tissue was present and similar in appearance to group 2. There was an increase in fibrosis of the operative axilla. In the fourth group sprayed with 1 to 100 dilution of proflavine hemisulfate, ten animals failed to exhibit tumor nodules at the end of 30 days. However, all axillae contained gross tumor pigment which was similar to the other groups. There was an increase in fibrosis and ulceration in this group. In the saline control animals, eight out of ten animals developed gross tumor nodules. Two animals failed to develop tumor or pigment staining in the axillary contents. From these pilot studies it is seen that using 1 to 1,000, 1 to 500, and 1 to 100 dilution of proflavine hemisulfate that tumor nodules did not develop at the end of 30 days. However, residual tumor cells remain on microscopic examination. Treating the inoculated axillae with sterile saline spray, a small percentage of animals failed to develop tumor or pigment staining axillae.

Significance to Cancer Research:

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As further laboratory and clinical experience with local chemotherapy of seeded operative wounds is obtained, it becomes apparent that more is involved in implantation and growth of tumor implants than the simple presence of the cells. The ability of implants to grow can be influenced in both a positive and a negative way. Simple application of tumoricidal agents is apparently not sufficient to control local recurrence of cancer. Possibly the most significant factor of these studies is the demonstration of a pre-treatment accentuation effect. If it were possible to understand the factors involved in this phenomenon, a means might be available for control of cancer in man.

Proposed Course of Project:

Continued efforts will be made to find an effective method of preventing local wound implantation of free cancer cells. The studies will probably lead to more basic understanding of factors associated with implantation and growth of cancer cells.

Part B included Yes [X] No [ ]

NCI-C-734
Serial No.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Heverback, Chester Z. and Smith, Robert R.: Transplantation of Tumor by Suture Thread and Its Prevention: An Experimental Study. Cancer 12:5, 1029-1042, 1959.

Honors and Awards relating to this project: None

Serial No. NCI-B-755(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Use of a Chemically-Defined Water Soluble Diet in Cancer Patients.

Principal Investigator: Dr. Robert B. Couch

Other Investigators: Dr. Leon Rosenberg, Dr. Donald Watkin, Dr. Robert R. Smith, Dr. Milton Winitz, Dr. Sanford Birnbaum, Mr. M. Clyde Otey, and Dr. Jesse Greenstein

Cooperating Units: Laboratory of Biochemistry and Metabolism Service, NCI

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1959):

Project Description:

Objectives:

The purification and synthesis of amino acids, carbohydrates, fats, and vitamins has allowed the nutritional investigator considerable freedom of manipulation of these essential nutrients and has aided in maintaining constancy of diets. Liquid diets, by providing uniform intake, enhance the quantitative validity of nutritional investigation. Greenstein and associates have added still another advantage to quantitative studies by designing an entirely water soluble diet. This insures equal exposure of all nutrients to intestinal mucosa as well as making diet analysis more accurate. These investigators have utilized the advantages of a chemically defined water soluble diet for quantitative nutritional studies in rats. Their investigation has been focused primarily on the amino acid content, and comparisons of ratios, quantities, and composition of this fraction have provided many illuminating features of amino acid metabolism. This project was designed in order to expand to humans these studies as well as numerous others. This report constitutes selection and study of a form of this diet suitable for clinical investigation.

Methods Employed:

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I. Design of Diet:

All vitamins and minerals used in this diet were felt to be essential for man. The mineral salts used were selected for solubility reasons. All quantities used were those recommended by the food and nutrition board of the National Research Council. Where no recommendation was available the literature was consulted and a reasonable quantity arrived at. Four mixtures of amino acids were used (Table I). Mixtures 1 and 2 were used in the preliminary studies and mixtures 3 and 4 in the metabolic balance studies. Mixture 1 includes all ten amino acids essential for the rat. Arginine was added because of the reported value in preventing ammonia toxicity. Histidine was included because only short term human studies have been performed without this amino acid. In preliminary studies DL Alanine was used as a source of nonessential nitrogen to make a total quantity of 75 grams of amino acids with a calculated nitrogen value of 11.2 grams per day. Mixture 2 was used in one patient. Mixture 3 was the basic mixture for balance study and consisted of the 18 amino acids present in casein. The ratio of essential amino acids used were those resulting from determination of the minimum requirement of each essential amino acid for 120 gram rats feeding solely on this type diet. The concentration of essential amino acids, 37% of the total quantity, was shown to produce optimal growth in the rat. The total quantity of each essential amino acid given per day was greater than the minimum intake determined by Rose. Ediol* was used as a source of fat in three instances to provide 1/3 of calories. This was later changed to ethyl linolenate, thus providing all nutrients in chemically defined form. The total lipid was the only nutrient in this diet present in less than optimal quantities. Dextrose, sucrose, and maltose were all used as a carbohydrate source. The only difference noted was the difference in relative sweetness when given orally. Dextrose was selected as the most desirable source of carbohydrate. The total daily diet aliquot provided 40 calories per kilogram weight except as noted.

II. Preparation and Administration of Diet:

All amino acids and monocalcium fructose 1,6-diphosphate were purified by dissolving in boiling water or hot alcohol, filtering through activated charcoal, and reprecipitating with [illegible] alcohol or vacuum distillation. The precipitate was filtered and washed with alcohol and ether. Sugar, vitamins, and minerals used were all of the highest purity obtainable. Ediol was obtained from Schenlabs Pharmaceuticals, Inc. Ethyl linoleate was obtained from the Hormel Research Institute and was 99% pure.

* Commercially available coconut oil mixture.

The diet was prepared in two ways. For the preliminary studies the chemicals were weighed and bottled in dry form in daily aliquots. These were put into solution in a Waring blendor and filtered through Whatman #4 filter paper. The sugar was placed in solution separately and the two solutions mixed. For the patients receiving Ediol, daily aliquots were bottled and the fat and fat soluble vitamins added on the day of use. In the remaining preliminary studies the ethyl linoleate and fat soluble vitamins were mixed thoroughly with polysorbate 80, then mixed with the diet to produce an emulsion. Daily aliquots were measured volumetrically and refrigerated until used.

For the balance studies the less soluble amino acids were placed in solution by shaking a 5 gallon jug containing an aliquot of distilled water. The more soluble amino acids, minerals, and vitamins were then placed into solution in a similar manner. Dextrose, dissolved separately, was added to the solution and shaking was continued until the solution was water clear. The diet was filtered through #4 filter paper, and ethyl linoleate and fat soluble vitamins added in the same manner as noted above. Daily aliquots were removed and frozen until 12 hours prior to use. They were then thawed, 200 cc. of 2% carboxymethylcellulose and flavoring (when used) were added, and the aliquot diluted to a predetermined 20 to 25% diet solution. Five separate feedings were measured out and refrigerated until given.

The diet was given in 12 separate courses to 7 patients. Five patients received the diet via indwelling esophagostomy tube and two patients received the diet orally. When given orally, flavoring was given to improve taste. Patients in the preliminary study received the diet in 3 to 8 separate feedings, and the two patients during metabolic balance study received the diet in 3 separate feedings per day. The feeding was taken over a 10 to 30 minute period. Distilled water was provided ad lib except during metabolic balance study where a regimented intake was used.

III. Design of Study:

1. Preliminary studies: Six patients received the diet for 20 days with 14 days of control before and after each experimental period. Two patients received the diet for 7 days only after the observation was made that all problems arose within this time limit. Weight was taken accurately every other day. A record was kept of each stool with its description and stool weights and volumes were measured on one male patient during the experimental period. Intake and output records were kept on five patients. Urine and blood studies were performed once weekly and included routine urinalysis, complete peripheral blood counts, and a broad coverage of blood chemistries.

Control diet was regular hospital or Waring blendor diet. Two patients received 30 calories per kilo during the control periods and one patient on a weight reducing regime received 1200 calories per day.

2. Variations in calories and amino acid isomers: Patient E.C. was selected for this study. Control diet was Waring blendor. Each study consisted of two 6 day periods with two or three control periods before and after each. Three forms of the diet were compared in this setting. The diet as listed with amino acid mixture number 3 at 40 calories per kilo, the same diet with added dextrose to make 50 calories per kilo, and the diet as listed with amino acid mixture number 4 were used in separate 12 day study periods. All diets, experimental and control, were isonitrogenous. All were isocaloric except as noted. Standard metabolic balance techniques were used. Diet and pooled urines and stools were analysed for total nitrogen, uric acid, K^+, Na^+, Cl^-, Ca^{++} and P^{+++}. Bloods were drawn and analysed one per period for TBS, BUN, creatinine, uric acid, ammonia, TSP A/G, Na^+, K^+, Cl^-, CO_2, Hct, Hgb, and WBC. Serum lipids consisting of cholesterol (total and esters), phospholipids, triglycerides, and unsaturated fatty acids were performed once per period. Theoretical and actual balance were calculated and results of each period compared.

3. Depletion-Repletion: Patient J.G. was selected for this study. Following a 12 day control period he was placed on a chemically defined water soluble nitrogen free diet for 12 days of nitrogen depletion. This was followed by 12 days of nitrogen repletion utilizing the same diet with amino acid mixture number 4. All diets were isocaloric throughout (40 calories/kilo), and the repletion diet and control diets were also isonitrogenous. Standard metabolic balance techniques were used and the same data obtained as above.

Patient Material:

These studies were performed on seven patients of the surgical service. An effort was made to select them at a time when no extraneous influences would occur during the study period. Other than this, the only criterion for selection was that they be stable in their disease process, cooperative, and have an intact GI tract and normal liver and renal function studies. A brief case history of the two patients on whom metabolic studies were performed will be described.

Patient E.C. (45 CM): Admitted to NIH in 1954. This patient gave a history of primary lip carcinoma in 1945 with wedge resection followed by irradiation of a recurrence. There was no

further treatment until 1954 when he presented himself at the NIH with extensive local involvement and bilateral cervical node metastases. A radical local excision with bilateral neck dissection was done. Since that time he has had numerous excisions for local recurrences. His weight has been stable. At the time of this study he had extensive local recurrence of his cancer associated with a great deal of pain. There was no evidence of distant metastases.

Patient J.G. (69 WM): This man presented himself at the NIH in 1957 with 4 x 4 cm. fungating epidermoid carcinoma of the hard palate. A radical local excision was done. Since then his weight has been stable and no evidence of recurrence has been noted.

Major Findings:

I. Preliminary Studies:

1. Clinical findings: All three patients receiving Ediol as a source of fat complained of occassional abdominal cramps. This has not been present since it was eliminated. Excess flatus and eructation were frequently reported, though clinically insignificant symptoms. Preliminary observations limited the difficulty to two symptoms, nausea and diarrhea. To elucidate their origin a series of elimination diets were set up designed to point to the cause of these symptoms. The sugar used in each diet bore no relationship to symptoms except that with sucrose the diet was reported as "nauseously sweet." Schedule of administration, Ediol, polysorbate 80, and D amino acids bore no relationship to nausea or diarrhea. The paramount factor as regards nausea proved to be diet concentration. Diet solutions of 35-65% produced nausea in four of five patients. Diet solutions of 20-25% failed to produce any nausea in three patients. When nausea did occur, an apparent physiological adjustment took place and the symptom would leave after 5 to 7 days.

Diarrhea could be more appropriately labeled "frequent call to stool" as measured volumes were always less than 200 cc. and weights less than 200 grams per 24 hours. However, it was an annoying symptom. On many days the patients would have 5 to 7 stools and in one patient as many as 12 per day. All stools were mucoid and small, and this symptom remained present throughout the experimental period. No specific cause has been discovered; however, when carboxymethylcellulose was added to all the special diet of one patient, diarrhea did not occur.

2. Weights: Two patients lost weight throughout the course of the study, one on 1200 calories and one in a prolonged postoperative state. These weight records are not included here. The two patients

receiving the diet for seven days only did not lose weight. The four cases presented here had no clinical or laboratory reason to lose or gain weight apart from the use of this diet. Patient E.C. had a steady gain in weight throughout the study with a total of one kilogram gain for the 48 day period. Patient M.C. had no change throughout. Patient M.F. lost 1.0 kilograms in the control period in association with a local surgical procedure. Following stabilization, the special diet was begun and she had no further weight loss. Patient J.G. lost 1.0 kilogram over the initial seven days of the experimental period. This was associated with clinically significant nausea. The regime was not changed, nausea disappeared after seven days, and he had no further difficulty. This was followed by regain of the weight loss.

3. Others: Intake and output records revealed no abnormal water balance. All of urine and blood analyses remained essentially unchanged throughout the 48 day period with the exception of blood urea nitrogen. This chemistry decreased during the experimental period in 5 of the 6 patients. Decreased urea synthesis has been suggested by the decreased urea excretion reported by Birnbaum with the use of a DL amino acid diet.

II. Variation in Calories and Amino Acid Isomers:

Patient E.C. was the subject. There was no significant change in weight or abnormal water balance. The patient experienced nausea with 25% diet concentrations requiring complete cessation of intake for 4/5 of one day. On decreasing concentration to 17-20%, no further nausea occurred. Whereas normally the patient had 1 to 2 stools per day, while receiving the experimental diet he had 2 to 3. Otherwise no clinical abnormalities were noted.

Balance data are as yet incomplete; however, nitrogen balances are for the most part available and are included in Table II. The very low stool nitrogen output was a feature common to all periods on the experimental diet. The asterisk marked periods were each accompanied by two days of nausea at the onset of the period. This nausea and diet adjustment may partly account for the low nitrogen retention values as compared to control periods. One cannot say that any of these diet mixtures are poor. Considering only the nitrogen balance data, it appears that the L amino acid mixture at 30 calories per kilo is equal in value to the control diet at 40 calories per kilo, whereas the L amino acid diet at 40 calories per kilo clearly seems to be inferior to the control diet. This is in keeping with the report of Birnbaum, et al., that though this diet is superior to all other mixtures used in their studies, it is still inferior to whole foods. This also supports the conclusion of Rose that in order to produce nitrogen retention, pure

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amino acid diets require a greater calorie per kilo value than isonitrogenous whole food diets. The diet containing racemic amino acids was clearly inferior to any of the others. Clarification of these conclusions awaits the complete balance data.

III. Depletion-Repletion:

Patient J.G. was the subject. There was no significant change in weight even during the depletion and repletion phases. Apart from urgency to stool and increase in frequency of 1-2 stools per day, the only symptom was that of mild lassitude during depletion which the patient was not aware of until he noted the difference during the repletion phase. The balance data given in Table II clearly show that the water soluble diet is capable of adequately repleting a human following nitrogen depletion. While total repletion during that phase of the study was not complete, the patient did not replete faster on a whole food diet. Again, clarification of these results awaits the complete balance data.

Significance to Cancer Research:

These studies provide baseline information and data on an experimental tool that can be used to ascertain the nature of amino acid and nitrogen metabolism in cancer patients. Ascertaining these abnormalities not only will further our understanding of cancer, but may lead the way to potential avenues of therapy.

Proposed Course of Project:

Publication of the data presented is planned. Further studies in the metabolism of cancer will await return of the senior author from temporary leave of absence.

Part B included: Yes [ ] No [XX]

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TABLE I

Diets Utilized in This Study

Diet 1

Vitamins:	Thiamine HCl	1.2	mgm
	Riboflavin	1.8	mgm
	Pyridoxine HCl	2	mgm
	Niacin	12	mgm
	Inositol	1	gm
	Choline HCl	150	mgm
	Ca. Pantothenate	10	mgm
	Biotin	1	mgm
	Folic Acid	2	mgm
	Ascorbic Acid	75	mgm
	Vitamin B_{12}	2	mgm
	PABA	500	mgm
	Vitamin A	4000	i.u.
	Vitamin D	800	i.u.
	Tocophenol	30	mgm
	Vitamin K	2	mgm
Minerals:	Potassium Citrate	12	gms
	Magnesium Citrate	0.25	gms
*	Potassium Hydroxide	2.4	gms
*	Magnesium Oxide	0.45	gms
*	Glucuronolactone	10.8	gms
	Potassium Iodide	0.3	mgm
	Manganese Acetate	22	mgm
	Zinc Benzoate	2.5	mgm
	Cupric Acetate	3	mgm
	Cobaltous Acetate	2	mgm
	Ammonium Molybdate	0.5	mgm
	Ferrous Ammonium Sulfate	84	mgm
*	Ferrous Gluconate	0.16	mgm
	Sodium Chloride	5	gms
	Monocalcium Fructose 1,6-Diphosphate	50	gms
Carbohydrates:	Dextrose		
*	Sucrose	remaining	
*	Maltose	calories	
Fats:	Ediol	1/3	calories
*	Ethyl Linoleate	2	gms

[illegible]
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Amino Acids:	DL Tryptophane	1	gm
	DL Phenylalanine	4.4	gms
	DL Threonine	2	gms
	DL Valine	3.2	gms
	DL Methionine	4.4	gms
	L Isoleucine (	2.8	gms
	D Alloisoleucine (		
	L Lysine	1.6	gms
	L Leucine	2.2	gms
	L Arginine HCl	2	gms
	L Histidine HCl	1.5	gms
	DL Alanine	50	gms

Diet 2

Vitamins: Same as Diet 1

Minerals: Same as Diet 1

Carbohydrate: Maltose

Fat: Ethyl Linoleate

Amino Acids:	L Tryptophane	0.5	gm
	L Phenylalanine	2.2	gms
	L Threonine	1.0	gm
	L Valine	1.6	gms
	L Methionine	2.2	gms
	L Isoleucine	1.4	gms
	L Lysine HCl	1.6	gms
	L Leucine	2.2	gms
	L Arginine HCl	2.0	gms
	L Histidine HCl	1.5	gms
	L Alanine	50	gms

Diet 3

Vitamins: Same as Diet 1

Minerals: Same as Diet 1

Carbohydrate: Dextrose

Fat: Ethyl Linoleate

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		% Total Nitrogen
Amino Acids:	L Lysine HCl	19.16
	L Histidine HCl	27.08
	L Arginine HCl	32.16
	L Tryptophane	13.72
	L Phenylalanine	8.48
	L Leucine	10.68
	L Isoleucine	10.68
	L Threonine	11.76
	L Methionine	9.36
	L Valine	11.96
	L Alanine	15.72
	Glycine	18.66
	L Aspartic Acid	10.52
	L Proline	12.17
	L Monosodium (	
	L Glutamate (	8.28
	L Glutamine	12.33
	L Tyrosin Ethyl Ester HCl	5.70
	L Serine	13.33
	L Cysteine Ethyl Ester HCl	7.54

Diet 4

Vitamins: Same as Diet 1

Minerals: Same as Diet 1

Carbohydrate: Dextrose

Fat: Ethyl Linoleate

Amino Acids: Same amino acids as Diet 3. All isomers were racemic DL form in twice the value given for essential amino acids and the same value for nonessential amino acids with the exception of those listed. These amino acids were L form and were used in the same quantities as in Diet 3.

L Lysine HCl
L Histidine HCl
L Arginine HCl
L Aspartic Acid
L Proline
Monosodium L Glutamate
L Tyrosin Ethyl Ester HCl
L Cysteine Ethyl Ester HCl

TABLE II

Nitrogen Balance Data

A. Variations in Diet

Period	Control		L as at 40 cal/kg		Control			L as at 50 cal/kg		Control		DL as at 40 cal/kg		Control
Average Daily Nitrogen Intake	10.32	10.32	10.32	10.32	10.86	10.86	10.86	8.88	10.25	11.03	11.03	11.10	11.10	10.20
Average Daily Stool Output	1.09	1.22	.33	.58	1.34	1.06	.85	.84	.60	.74	.72		1.27	.17
Average Daily Urinary Output	7.19	7.62	9.18	8.21	6.82	6.98	8.72	6.62	6.80	7.36	7.85	11.60	7.78	6.64
Daily Nitrogen Balance	+2.04	+1.48	* +.80	+1.52	+2.70	+2.82	+1.29	* +1.48	+2.85	+2.94	+2.46		+2.04	+3.39

B. Depletion-Repletion

Period	Control		Depletion		Repletion		Control	
Average Daily Nitrogen Intake	11.13	11.13	.26	.25	10.93	10.93	11.04	11.04
Average Daily Stool Output	1.23	1.98	.48	.66	.59	.98	1.31	1.22
Average Daily Urinary Output	8.50	7.31	3.85	2.72	7.42	7.06	7.16	7.84
Daily Nitrogen Balance	-1.40	+1.83	-4.07	-3.14	+2.92	+2.89	+2.56	+2.78

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1. Surgery Branch
2.
3. Bethesda, Md.

DHEW-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Viruses Associated with Human Tumors.

Principal Investigators: Dr. Robert A. Manaker, Laboratory of Biology, NCI, Dr. Robert B. Couch, and Dr. Edward McLaughlin

Other Investigators:

Cooperating Units: Laboratory of Biology, NCI

Man Years (calendar year 1959):
Total: 1½
Professional: 1
Other: ½

Patient Days (calendar year 1959):

Project Description:

Objectives:

Studies have demonstrated that viruses may be associated with animal tumors in any of several ways. The virus may be only a passenger serving no beneficial or detrimental relationship with the tumor. It may be a latent infection of varying duration, activity when it occurs being manifested by necrosis of tumor or stimulation of growth. Secondarily infected tumors by artificial or natural means may result in tumor necrosis and finally, viruses infecting normal tissue with or without an intervening latent state may induce tumor formation in this tissue. Many of these relationships have been demonstrated in virus-animal tumor systems. Some have also been demonstrated in virus-human tumor systems. In order to study these virus-human tumor relationships, cooperative studies were instituted between the surgical branch of the NCI and the other units of the NIH. The present study is a cooperative venture between the Laboratory of Biology and the Surgery Branch of the NCI. It has as its objective the isolation of viruses from human tumors and the characterization of the relationship that existed. The effort of the surgical branch has been directed by the premise that the virus might be unmasked and/or produced in sufficient quantity for study by maintaining the parent tumor in a host free system. This system has consisted of tissue cultures of the tumors in heterologous or artificial media.

A brief description of the project is included (Table I)

along with complete details of that part of the project carried out by the Surgery Branch.

Materials:

1. Hanks Basal Salt Solution (wash fluid)
2. NCTC 109 Medium
3. Eagle's Medium
4. Yeast Extract Medium
5. Chang's Medium
6. Pooled Human Serum (contract)
7. Horse Serum
8. Penicillin and Streptomycin
9. T-15 Culture Flasks with and without Perforated Cellophane

Methods:

1. The tumor immediately after excision is taken to Pathology where it is cut under sterile conditions and a portion placed in chilled basal salt solution in an ice bath. When possible, a larger portion of the tumor is taken after photography and placed in a separate container. The first portion is used for T-15 flask cultures and overlaid on indicator cell lines. The larger portion is frozen for extraction and concentration.

2. An approximately 1 x 1 x 1/2 cm. wedge of tumor is minced finely with iris scissors until no further reduction in size of bits can be obtained. The material is then washed three times with chilled Hanks Basal Salt Solution with centrifuging and discarding of supernatant fluids between washes. The final button of tumor is used for inoculating T-15 flasks and indicator cell lines.

3. With a Pasteur Pipette the tumor is transferred from the centrifuge tube to each of four T-15 flasks where it is equally distributed. Then 3 ml. of 10% Human 109 media containing Penicillin, Streptomycin, and 40% serum is added to each of two flasks.

4. The T-flasks are incubated at 37° C. Observations are made three times weekly and fluids replaced, saved, or changed, depending upon what is observed. At the end of four weeks, if no growth has occurred, the T-flasks are terminated. If growth occurs, this is carried to full spread over the T-flask surfaces split with a portion of the tumor being homogenized, passed to indicator cell lines, and a portion carried by passage. If any spontaneous degeneration of tumor or cytopathogenic effect is seen

TABLE I

Outline of Procedure for Processing a Specimen

1	2	3	4 Fate
Tumor	Mince	T-Flasks	a) Fluids on degenerating tumor passed to indicators and passed there X4 at approximately 4-week intervals. b) Growth passed to indicators and passed there X4 at approximately 4-week intervals.
		Indicators a) Skin b) Bone c) Fibroblasts d) Intestine	Passed at approximately 4-week intervals.
	Extract	Indicators a) Skin b) Bone c) Fibroblasts d) Intestine	Passed at approximately 4-week intervals.
		Animals	Injected with extract and followed for tumor formation or other evidence of disease.

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in the tissue culture, fluids and/or cells are saved for inoculating on indicator lines.

Patient Material:

42 tumors have been utilized in this study. All were removed at surgery from patients occupying beds of the Cancer Institute. Only primary tumors were used and no other criteria for selection of tumors was invoked.

Major Findings:

The first 19 tumors excised were planted in T-25 flasks with several types of media used and both human and horse serum. Serum was used at 10% concentration. Spindle cells were grown in almost all instances with tumor cells in none. Starting with tumor #20, 40% serum was used and tumor growth was obtained. This summary is of the last 23 tumors cultured (see Table IX).

Media

1. 40% Human Serum NCTC 109: Growth in 1 of 5 tumors. This media has been dropped.

2. 40% Horse Serum NCTC 109: Growth in 12 of 14 tumors. This media has been maintained as one of the permanent medias.

3. 40% Human Serum Eagles: Growth in 11 of 14 tumors. Spontaneous degeneration was not uncommon so the advantages of this media were combined with YEM.

4. 40% Horse Serum Eagles: Growth in 7 of 10 tumors. This media has been combined as was 40% human Eagles.

5. 40% Human Serum Yeast Extract: Growth in 7 of 9 tumors. It was difficult to maintain alkaline pH so this media has been combined with Eagles.

6. 40% Horse Serum Yeast Extract Medium: Growth in 7 of 9 tumors. Combined as was 40% horse Eagles.

7. 40% Human Chang: Growth in 2 of 2 tumors. This media was dropped because chick embryo extract was employed. There is theoretical objection to viruses possibly being present in the extract.

8. 40% Horse Serum Chang: Growth in 1 of 3 tumors. This

TABLE II

Growth Result of Human Tumors Inoculated in T-15 Flasks
By Media Employed

Tumor Number	109 Hu	109 Ho	E Hu	E Ho	Y Hu	Y Ho	C Hu	C Ho
20	0	0	+	0	+	0	+	0
21	0	0	+	+				
22	+	+	+	+	+	+	+	+
23	0	+	+	+				
24	0	+	+	0	0	0		
25			0	?			?	0
26	?	?	?	?	?	?	?	?
27		+	0	0				
28		+	+	?				
29		?	+	?	+	?	?	?
30		?	?	?	?	?	?	?
31		?	?	+				
32		+	+	+	+	+		
33		+	+	+	+	?	?	?
34		+	0	?	?	?		
35		+	+	?	+	?		
36		?	+	+	+	?		
		109 Ho	E Y Hu	E Y Ho				
37		?	+	+				
38		?	?	?				
39		+	+	+				
40		+	+	+				
41			+	?				
42		+	+	+				

Key: 0 = no growth
+ = growth of tumor cells
? = contamination before a result obtained

medium has been dropped for the same reason as human serum Cheng.

9. 40% Human Serum 3/4 Eagles' and 1/4 Yeast Extract Media: Growth in 5 of 5 tumors. This is retained as one of the permanent medias.

10. 40% Horse Serum 3/4 Eagles' and 1/4 Yeast Extract Media: Growth in 4 of 4 tumors. This is retained as one of the permanent medias.

Cell Type: Growth was accomplished in tissue culture for 10 of 10 epidermoid carcinomas, 4 of 5 adenocarcinomas, and 4 of 4 miscellaneous type tumors. Not all medias were successful in producing growth. 4 tumors were overgrown with contaminant before growth or failure of growth could be ascertained. This leaves 19 tumors for observation; 18 of these tumors grew (see Table III).

Effects in Tissue Culture: Cytopathogenic effect has been seen in the cell sheet of 4 tumors. The cell type was epidermoid in 3 and undifferentiated in 1. Spontaneous degeneration of the cell sheet has been seen in 3 tumors. The cell type was epidermoid in 2 and adenomatous in 1. Fluids and cells were saved on all these for inoculation on tissue culture indicator lines. No evidence of virus has been seen in the indicator lines.

Discussion: The techniques employed to establish tissue culture appear to be adequate for the purpose. The primary problem has been that of fungus contamination of cultures. It is apparent that occasionally the tumor, itself, is contaminated with fungus to grow inherent. However, the loss of established tissue cultures to fungus contamination has been staggering. All media, media supplements, glassware, and instruments were checked for fungus and were negative. Air pollution seems to be the most likely source. Ultraviolet light has failed to control the problem so a hood for isolation of the work area has been ordered.

Significance to Cancer Research:

With the current popularity of the theory that viruses are the etiological agents of human cancer, study of the virus-tumor relationship in humans is fundamental to the understanding of cancer.

Proposed Course of Project:

This study is being continued. In absence of Dr. Grace [illegible]

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TABLE III

Results by Cell Type of Tumor Inoculated

Cell Type		Growth/Number of Tumors
Epidermoid Cancer		10/10
Adenocarcinoma		4/5
Miscellaneous Cancers		4/4
	Total	18/19

NIH, Dr. Edward McLaughlin is continuing to grow primary cancers excised at surgery on tissue cultures and to supply Dr. Mansher and Dr. Sarah Stewart with some of the same material for virus culture studies.

Part B included: Yes [ ] No [XX]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental Studies of Mechanisms of Metastases; Effect of Surgery, Radiation, Removal of Primary Tumor, and Adjunctive Chemotherapy on Incidence of Artificial and Spontaneous Lung Metastases in Experimental Animals.

Principal Investigators: Dr. Alfred Ketcham and Dr. Robert Smith

Other Investigators: Dr. David Kinsey, Dr. Robert Hoye, Dr. Alvin Harris, Dr. John Dillon, Dr. Edward Price, and Miss Hilda Wexler

Cooperating Units: Laboratory Pathology

Man Years (calendar year 1959):
Total: 3
Professional: 2
Other: 1

Patient Days (calendar year 1959):

Project Description:

This project is made up of a number of parts by a group of individual investigators.

Objectives:

1. To develop laboratory models which would simulate the metastatic process in humans. This includes studies to determine the time of metastases in mouse tumor systems.

2. To identify and measure factors which influence the spread of cancer.

3. To measure the effects of adjunctive chemotherapy and immediate preoperative radiation on the incidence of artificial and spontaneous metastases.

Methods Employed:

Part I. Standardization of Cloudman S-91 Melanoma, [illegible] Sarcoma, T-241 Sarcoma, Amelanotic S-91 Melanoma, and Osteogenic Sarcoma 112 (Dr. Ketcham)

These tumors are all being used in the laboratory of the Surgery Branch for various investigative animal studies. In these tumors studied, the effect of surgical removal of a "primary tumor" on development of lung metastases is determined by counting the

number of metastases under standard conditions of time, surgery, housing, inoculum, etc. Each of the tumors is carried in the hind leg of a mouse--the melanoma tumors in the CDBA animals, the DBA-49 tumor in the DBAF mice, the T-241 in C_{57}, and the osteogenic sarcoma in C_3HF_1. Inoculums are all similarly prepared by sacrificing the donor animal, taking a weighed amount of viable tumor tissue, passing it through a Snell cytosieve with a 1:10 dilution of saline, and using .05 cc. of the inoculum in the hind leg of the appropriate mouse to be inoculated.

The longer the animals can survive in the tumor bearing state, the more significant the results of the experiment. It has been found that individual housing of these animals allows an extended life span presumably due to the lack of cannibalism and the ability of the tumor laden mouse to more adequately obtain food and water.

The nonpigmented tumors studied are most difficult to adequately evaluate if one attempts to count the lung metastases with the naked eye or without some type of staining technique to bring out these tiny tumor foci. Miss Wexler has developed a technique of India ink dye insufflation of the lungs whereby even tiny microscopic foci of tumors are identifiable and not only countable but able to be categorized into size comparisons. Confirmation of the adequacy of this 15% India ink insufflation technique has been shown by microscopic sectioning of these lungs showing tiny foci of tumor in the area occupied by the dye.

It was found necessary to compare studies to determine the effect of removal of a "primary" tumor by amputation on development of lung metastases with controls living the same length of time. Three tumor systems, S-91 Cloudman melanoma, DBA-49, and T-241, were employed in a manner similar to that previously discussed. All animals had their tumor bearing leg amputated when "the primary" was 1.5-2 cm. in size. They were individually housed and then sacrificed at intervals: in the S-91 series at 4, 6, 8, 10, and 12 weeks postinoculation; in the DBA-49 series at 3, 5, 6, and 7 weeks postinoculation; and in the T-241 mice at 17, 24, 31, and 38 days postinoculation. The lungs were counted and comparisons made.

In another study, the effect of partial removal of a "primary tumor" on development of lung metastases was determined. Using two tumor systems, S-91 and DBA-49, the animals were divided into three groups when the tumor growing in the leg reached the amputation size of 1.5-2 cm. One third of the animals were left untouched with their tumor allowed to grow. One third had their tumor incised under intraperitoneal nembutal anesthesia and

essentially 90%+ of the tumor tissue curetted from the leg. The wound was then closed with skin clips. The third group was similarly anesthetized and had complete amputation of the primary tumor. These animals were followed to the previously determined sacrifice date and then comparison counts of lung metastases made.

Part II. (Dr. Hoye)

This study was designed to determine if amounts of irradiation which were too small to have any measurable effect on the growth of a "primary" tumor were effective in altering the component cells of this tumor so as to prevent their growth as lung metastases when surgically detached and artificially disseminated as tumor emboli.

Four tumor-host systems in female mice eight weeks of age were used in this study: the Cloudman S-91 melanoma in DBA/P and in CnDBA/2F_1 mice; the Sarcoma 49 in DBA/P mice; and the Sarcoma T-241 in C_{57} BL/6JN mice. All four systems in this laboratory demonstrate spontaneous metastasis to the lungs. The design of the experiment as used with each tumor-host system consisted of inoculating a group of twenty-four mice in the hind leg with a suspension of tumor cells. The tumor inoculation was prepared by cytosieving approximately 0.8 grams of freshly excised tumor in 8 ml. of normal saline. All tumor cell suspensions were prepared in the same manner and a few heparin crystals were added to reduce the tendency of the cells to clump and give a more even cellular suspension for intravenous injection. A suspension so prepared contained approximately 150,000 cells per each injection.

In approximately two weeks the transplanted leg tumor, hereafter referred to as the "primary" tumor, had grown to a size of 10-12 mm. and was still in an actively growing phase. The twenty-four mice were then divided into four groups with six animals in each group. One group served as controls while the other three groups received irradiation to the "primary" tumor. Irradiation was accomplished with a 200 KV Westinghouse machine with physical factors enabling one to deliver a tumor dose of 341 r per minute. During irradiation the tumor was exposed, while the remainder of the animal was shielded with lead. Amounts of x-ray administered to the three treatment groups in each tumor-host system were 170 r, 715 r, and 2000 r. These amounts were chosen as preliminary studies with S-91 in CDBA mice. They had demonstrated that changes in the growth of detached cells was demonstrable following in vivo irradiation with amounts as small as 170 r. Gradually increasing doses demonstrated increasing effectiveness until 2000 r was reached. From 2000-4300 r there was no added effect.

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Twenty-four hours following treatment, one half of the animals in each group of six were sacrificed and a suspension of cells made from each tumor. Animals not sacrificed were followed daily to detect alterations in growth occurring in the transplanted tumor. In the sacrificed animals the viability of the cells composing the "primary" tumor was evaluated by injecting the cell suspension made from each tumor intravenously into four tumor free mice of the same strain. Growth of the intravascular inoculum occurred in the lungs as discrete tumors and growth in other sites was not observed. The amount of cell suspension injected intravenously and the day of sacrifice of the recipient animals varied in each tumor-host system. These values were determined in preliminary studies and depended upon the number and size of lung tumors occurring which would permit accurate counting. Following sacrifice of the injected animals, the lungs were fixed and the number of tumors counted with a 3-D magnifier. Pigmented tumors were easily visible and the nonpigmented tumors made so by prior staining of the lung with India ink.

Part XII. Effects of Nitromin (Dr. Dillon)

Toxicity Studies: Female CDBA mice were injected intraperitoneally with solution of Nitromin in saline at dosage levels ranging from 25 to 300 mg. per gram of body weight. Both single doses and multiple doses were tested.

Therapeutic Studies:

1. The effect of Nitromin in various concentrations on S-91 melanoma, when mixed with the tumor in the test tube, was determined. Equal volumes of 10% S-91 melanoma were mixed with saline and solutions of Nitromin ranging in concentration from 5% to 0.000000l%. The mixtures were then injected into the muscle of the hind limb of female CDBA mice. The diameter of the resultant tumors, if any, was then measured with calipers.

2. Female CDBA mice were given intravenous inoculation of cytosieved preparation of S-91 melanoma in saline (5%). Nitromin was then injected intraperitoneally and intravenously at various times following inoculation and in varying dosages. Both single and divided doses were used. Animals were then sacrificed and the number of lung metastases counted. The number of metastases was then compared with control groups which received no treatment and the differences between groups which differed with respect to time and dosage of treatment were compared as well as the effect of the route of administration.

3. Experiments are currently underway using the above

outline, using T-241 sarcoma in C_{57} black mice.

Part IV. Time Study of Metastasis (Dr. Romodahl)

1. DBA female mice bearing Sarcoma 49 tumor in the thigh are used. Mice are injected in the thigh intramuscularly on day 0 and amputations are done on a group of mice on days 1-15. All mice thus amputated are sacrificed on a later date and lung metastases counted.

2. Similar to course as described above using C_{57} female mice and T-241 tumor.

3. DBA female mice are injected with Sarcoma 49 and C_{57} female mice are injected with T-241 tumor intramuscularly on day 0. On days 1-14 two mice are sacrificed and their lungs removed. Lung fragments are divided and injected intraperitoneally into eight C_{57} female mice on each day. The animals will be allowed sufficient time to develop abdominal tumors and then will be sacrificed. Providing lung particles thus injected contain viable tumor cells or implants, it is possible that the finding of abdominal tumors may indicate more precisely the time of metastases in the previously sacrificed animal.

Part V. Studies with a Transplantable Hamster Melanoma (Dr. Harris)

Tumor was obtained originally from the Laboratory of Pathology (NCI) where it had arisen spontaneously in a hamster twenty months of age. Tumor had subsequently been transplanted via trocher technique through several generations primarily for maintenance of tumor. The tumor has been treated as follows: From a donor animal, 1-1.5 grams of viable tumor is dissected free and minced with 10 ml. of normal saline and passed through a cytosieve. The tumor solution is injected into male hamsters 4-5 weeks of age, either subcutaneously in the back or intramuscularly into a hind leg. The animals are then observed for development of tumor, growth characteristics, and finally patterns of metastases. 130 animals have been inoculated, with autopsy performed on all who have died or been killed (65).

Part VI. Studies of Preferential Metastasis S-91 to Lungs (Dr. Kinsey)

Numerous studies have shown that metastasis of S-91 melanoma was limited to lungs. Experiments were run in which cytosieved inoculum was injected into tail artery, vein, left heart carotid artery, and portal vein. Additional studies were

done with inoculation into animals that had received organ transplant of lung, kidney, liver, etc.

A time sequence study of pulmonary metastasis of S-91 melanoma was also performed. Mice were killed immediately (6, 12, 24, 36, and 48 hours; 3, 5, 10, and 21 days) after tail vein inoculation of cytosieved inoculum. A lobe of lung was removed at the stated time and serial sections of the tissue made. Careful separate study of these sections was then made by Dr. Rinsey and Dr. Edward Price (Pathology) noting the position of and growth of the embolic tumor nodules.

Major Findings:

Part I. (Dr. Ketcham)

Standardization of S-91 Melanoma: This tumor is now four and a half years old in this laboratory and still maintains its same biological characteristics of metastases limited to the lung. It requires, as shown by new tumors obtained from other laboratories within the institute, approximately 7 to 9 cytosieve transfers before it develops the ability to metastasize. This is in contrast to the tracer transfer of this tumor, whereby no metastasis is found.

Standardization of DBA-49: This tumor has been carried for two years in this laboratory and has remained consistent in its ability to develop lung metastases from the onset of transfer of the cytosieve inoculum. There has been no apparent biological change in its character. As with the other tumors, when the primary tumor in the leg reaches a size of 1.52 cm. it is amputated by the cautery clamp technique which brings about an essential 100% clean amputation without recurrent tumor growth locally. This is a more rapidly growing tumor, and with the individual housing the tumor bearing animals survive up to five weeks.

Standardization of T-241: In nine months' evaluation of this tumor, its biological characteristics have remained unchanged. It metastasizes primarily to the lungs, is rapidly growing, and allows for adequate counting of its lung metastases. It reaches the size of 1.5 cm. at approximately 10 days. The animals are sacrificed at 24 days.

Standardization of Amelanomic S-91: Preliminary studies with this tumor indicate that its growth requires amputation on the 31st day in order to prevent local regrowth. By the 35th day, tumor bearing animals start to die and thus this sacrifice date is chosen.

Standardization of Osteogenic Sarcoma: This tumor requires amputation on the 23rd day and sacrifice on the 47-52nd day.

Results of Amputation Studies: The number and sizes of lung metastases observed after amputation of a "primary" S-91 melanoma 28, 35, 42, and 49 days after inoculation in leg are shown in Table I. It shows a stepwise increase in the number of metastases with a lengthening of survival time of the controls to 49 days. There is a strong suggestion that there is an increase up to a certain period when a leveling off period is observed, but the sizes of the metastases continue to increase. Whether the metastases continue to shed tumor cells and set up separate additional foci of metastases can not be determined. The finding of continued increase in the size of the individual tumor in the absence of small metastatic foci does not support this belief. In the latter days of the experiment, the tumors within the lungs seem to coalesce and the animals develop cachexia and die in a manner similar to those who are allowed to progress with their primary tumor intact. Although the amputated groups of animals had smaller numbers of metastasis, the average size of the metastasis was greater than in the tumor bearing group. This lends support to the hypothesis that there is a constant shedding of tumor cells from the primary tumor, giving both large and small foci in the tumor bearing animals, but only large foci in those which had the primary tumor removed.

The animals involved in the experiments were usually six to eight week old mice. Several experiments were carried out comparing these young mice to six month old mice to determine if there would be change in tumor characteristics because of the age difference. Using the S-91 Cloudman melanoma in the CDF1 mice and DBA-49 in the DBAP mice, each age group had an amputated and a control group and were followed as in the previously discussed experiment. There was found to be no difference between age groups in either series in the numbers or sizes of metastases. The intact tumor bearing groups of both ages showed a greater spread of metastases than the amputated animals, and again the size foci were larger in the amputated groups. Conclusions and findings are similar when Sarcoma DBA-49, Sarcoma 241, osteogenic sarcoma, and S-91A were studied. In general there is an increased incidence of metastases and increase in numbers of metastases within the individual mouse in the nonamputated (tumor bearing) animals, as compared to those animals in which the primary tumor was completely removed.

Table II demonstrates that those animals who had tumor partially removed had a similar number of metastases and essentially

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TABLE I

Summary of the Spread of Lung Metastases, with Time, in Amputated and Control Animals Using Cloudman S-91 Melanoma

Sacrifice	Average of 3 Experiments	
	Amputated	Control
Day 28		
Med. No. Lung Tumors per Mouse	0	0
Med. No. Lung Tumors per Tumor Mouse	2.5	3.2
Mice with Lung Tumors, Percent	27	39
Size of Lung Tumor - Average	2.6	2.0
No. of Mice Employed	70	75
Day 35		
Med. No. Lung Tumors per Mouse	1.0	5.6
Med. No. Lung Tumors per Tumor Mouse	3.0	7.7
Mice with Lung Tumors, Percent	61	75
Size of Lung Tumor - Average	3.4	2.0
No. of Mice Employed	73	77
Day 42		
Med. No. Lung Tumors per Mouse	2.0	10.4
Med. No. Lung Tumors per Tumor Mouse	5.3	12.7
Mice with Lung Tumors, Percent	67	89
Size of Lung Tumor - Average	4.7	2.9
No. of Mice Employed	72	72
Day 49		
Med. No. Lung Tumors per Mouse	4.5	15.1
Med. No. Lung Tumors per Tumor Mouse	5.0	16.0
Mice with Lung Tumors, Percent	69	97
Size of Lung Tumor - Average	6.3	3.4
No. of Mice Employed	76	77

TABLE IV

The Effect of Incomplete Removal of a Primary Tumor upon Metastases

	Cloudman S-91 Melanoma			Sarcoma DBA-48			Sarcoma T-241		
	Complete Removal	Partial Removal	Intact	Complete Removal	Partial Removal	Intact	Complete Removal	Partial Removal	Intact
Tumors Per Mouse*	5.0	9.0	9.0	6.5	28.0	30.0	10.0	39.0	35.0
Tumors Per Tumor Mouse*	7.0	11.2	10.0	7.0	28.0	30.0	10.0	39.0	35.0
Per cent With Metastases	71%	87%	97%	90%	100%	100%	100%	100%	100%
Size of Tumor Foci	3.4	2.9	2.6	9.1	7.9	6.9	8.0	6.4	5.7
Number of Animals	52	51	51	30	29	29	23	22	21

* Median Number

identical sized range as those in whom the primary tumor was left intact. The completely removed tumor groups had less metastases, and again the average size was noticeably larger than that of the amputated and the partially amputated groups.

Part II. Effects of Radiation on Implantation of Cancer Cells (Dr. Hoye)

The effect which irradiation had on the growth of the "primary" tumor was manifested by an interuption in the growth curve following treatment ranging from 0-7 days and represented on Table III as a 1+ response, or a delay in growth of from 7-14 days and represented as a 2+ response. Following this delay the tumors resumed growth at a normal or near normal rate and eventually killed all of the animals. It is to be noted that in none of the four tumor-host systems used did complete cessation of growth occur nor did tumor regression take place.

The effect of irradiation on individual cells as manifest by the number of lung tumors produced following the inoculation of the in vivo irradiated tumor cell suspension was measured as the mean number of lung tumors per mouse from the twelve mice in each group (see Table III). This value varied considerably between the controls and each treatment group. While the best response in preventing growth of the disseminated cells was obtained following amounts of irradiation which also altered the growth curve of the "primary" tumor, this was not invariably true. The T-241 "primary" demonstrated no response to 715 r, yet no lung tumors occurred following injection of its suspension, as compared to 43 tumors per mouse in control animals. The Sarcoma 49 "primary" was not effected with 2000 r, yet this amount of radiation was effective in decreasing the number of lung tumors from 113 in controls to 22 tumors per mouse in the animals injected with the in vivo irradiated cell suspension.

Thus we see that in 3 out of the 4 tumor-host systems used a tumor dose of 715 r was effective in reducing the number of lung tumors at least 70%. When 2000 r was administered prior to artificial dissemination, the number of metastases was reduced by 81-100% of control values.

For many years the radiotherapy of cancer has been concentrated in attempts at killing the component cells of a malignant tumor with large doses of irradiation. Although radio-biologists have known for some time that small amounts of irradiation are capable of altering cellular physiology, this concept has not been applied clinically. The concept of [illegible]

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of malignant cells at surgery which subsequently produce local and distant recurrence of tumor is a well recognized one. This experimental study has demonstrated that small amounts of irradiation administered to these cells before detachment from the primary tumor occurs, are sufficient to prevent implantation and growth of these cells following detachment and dissemination.

The mechanism responsible for this change in growth potential of disseminated cells is not known. It is believed that the decreased in the mean number of lung metastasis is not due to the in vivo irradiation death of the inoculated cells, as death of such a large number of cells should produce a more marked alteration in the growth curve of the "primary" tumors. A temporary interruption of cell reproduction is possible, but in a group of animals injected with in vivo irradiated inoculum and sacrificed at five weeks instead of the usual three weeks, there were the same number of lung tumors, but the tumors were of larger size. This suggests that the added survival time did not permit recovery of more of the irradiated cells. The cells partially damaged by the in vivo irradiation may die from lack of nourishment supplied by the tumor bed, they may be unable to implant into the host tissues, or they may be more susceptible to natural host defenses.

Tumor and Host		Control	170r	715r	2000r
S-91 in DBA	* "Primary"	0	0	+	++
	** Response	314	265	66	3
S-91 in CDBA	"Primary"	0	0	+	++
	Response	440	263	133	2
S-69 in DBA	"Primary"	0	0	0	0
	Response	113	107	60	22
T-241 in C-57	"Primary"	0	0	0	+
	Response	43	30	0	0

TABLE III

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Part III. Nitromin Study (Dr. Dillon)

This study is still in progress. It is evident, however, that Nitromin when mixed in vitro with S-91 melanoma prevents its growth in concentrations of 1% or greater. At lower concentrations the growth of tumor was enhanced when compared with the growth of tumors which had been mixed with saline (this will become the subject of a later study). The effect of Nitromin in inhibiting artificial metastases of S-91 melanoma, however, is negligible. Intravenous Nitromin had a slight effect, but single intraperitoneal doses of Nitromin had almost no influence on such metastases. The results of divided doses is not yet known. Toxicity studies, however, indicate that a substantially greater total dose of Nitromin can be given in this manner. These studies indicated that the LD-50 of Nitromin in CBBA mice is approximately 120 mg./g., with 50 mg./g. being the maximum safe single dose intraperitoneally. The studies with T-241 sarcoma are much less complete but thus far are no more encouraging.

Part IV. Hamster Melanoma Study (Dr. Harris)

The purpose of the study was to attempt to achieve a standard system that could be used in the study of dynamics of metastasis. After the transplantable tumor had gone through 10 passages involving 96 animals, marked variations in numbers of lung metastases persisted and the pattern of metastatic growth remained quite variable. The life span of the tumor bearing animals did become more constant. Metastases were noted in 92% of the animals studied and were seen in lungs, hilar nodes, axillary nodes, spleen, and once in jejunum. Lung metastases were most constant, varying in numbers from 1 to 1100.

As with S-91 melanoma, the hamster melanoma seemed to lose its ability to metastasize when transplants were made by trocar. Cytocloved inoculum restored the ability of the melanoma to metastasize. Changing the site of inoculation from leg to back produced changes in the pattern and extent of metastasis.

Since hamsters are not a truly inbred strain of animal, and this system not isologous, the value of this tumor system in quantitative metastasis studies would seem to be questioned.

Part V. Study of Preferential Metastasis S-91 Melanoma to Lungs (Dr. Kinsey)

Following free grafts of various tissues into thighs of DBA/2 mice later given intravascular inoculation of S-91 melanoma, artificial metastasis developed only in the insitu lungs and in the free grafts of pulmonary tissue. This is summarized in the following table:

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Route of Inoculation	Transplanted Tissue	Number Animals	Number Animals with Tumor in Transplanted Tissues	Number Animals with Tumor in Insitu Lung
Tail Artery	Lungs	40	37	40
	Kidney	5	0	5
	Spleen	5	0	5
	Heart	5	0	5
	Skin	5	0	5
	Thyroid	5	0	5
	Control	5	0	5
Left Ventricle	Lungs	8	4	8
	Control	8	0	8
Tail Vein	Lung	8	2	8
	Control	8	0	8

Intraportal vein inoculum gave rise to liver metastases only when the amount of inoculum was enough to kill most of the animals. The conclusion of the study was that the lungs are truly a preferential site of metastasis for this strain of animal melanoma.

To determine the time relationship of pulmonary metastasis following intravenous inoculation of S-91 melanoma in the study, it was demonstrated that the tumor emboli were largely intravascular for the first 36-48 hours after inoculation. Until this time, the emboli were mostly single cells and small clumps, frequently surrounded by macrophages and debris. After 48 hours the implants were noted to be intravascular and growing. As time progresses, the intravascular emboli disappear first from smaller capillaries and later from larger pulmonary vessels. By 21 days no intravascular emboli are apparent.

The change in position of the emboli from an intravascular to extravascular position correlates with the degree of effectiveness

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of ThioTEPA in preventing growth of the implants (Kramer). This same correlation is noted in work of Warren and Gates using Walker carcinosarcoma 256 and by Woods using V_2 rabbit carcinoma.

Significance to Cancer Research:

If human cancer therapy is to be improved, an understanding of the factors that control metastasis must be found. Realising that any transplantable animal tumor is never exactly analogous to the situation found in human cancer, it is anticipated that a standardized animal model system would greatly help in establishing the value of any procedure. This may be especially true in adjunctive chemotherapy and radiation. The study represents an approach to the treatment of metastases during the period of their implantation and early growth, a situation analogous to the immediate postoperative period after cancer surgery.

Proposed Course of Project:

It is proposed to continue the study to determine factors that influence implantation and growth of tumor emboli. It is hoped that the effect of stress, infection, and trauma on metastasibility can be determined. A search for a more isologous system will be continued, and attempts to use spontaneous rather than transplanted tumors will be made.

In the study of the effect of radiation on dissemination of cancer cells, it is planned to continue with at least one additional tumor-host system, that of a mammary carcinoma. In addition, experiments are currently underway in which this in vivo irradiated inoculum is being injected intramuscularly and into an axillary wound. Partially evaluated have been the S-91 melanoma in CDBA mice and the T-241 sarcoma in C_{57} mice. While growth occurred in 100% of control mice, only 12% of the treated mice developed tumor.

It is conceivable that systemic chemotherapeutic agents might also produce a similar cellular alteration capable of preventing growth of the disseminated cells and this concept will be evaluated in a similar experimental system.

Part B included: Yes [XX] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Ketcham, Alfred S., Wexler, Hilda and Mantel, Nathan: The Effect of Removal of a "Primary" Tumor on the Development of Spontaneous Metastases. I. Development of a Standardized Experimental Technique. Cancer Research 19:9, 940-944, 1959.

Kinsey, David L. and Smith, Robert R.: Contrasts Between Human and Experimental Melanomas. Surg. Gynec. & Obst. 109:5, 539-543, 1959.

Olch, Peter D., Eck, R. V. and Smith, R. R.: An Experimental Study of the Effect of External Irradiation on a "Primary" Tumor and Its Distant Metastases. Cancer 12:1, 23-26, 1959.

Olch, Peter D., Eck, R. V. and Smith, R. R.: An Experimental Study of the Effect of Irradiation on the Dissemination of Cancer. Cancer Research 19:5, 464-467, 1959.

Honors and Awards relating to this project: None

Serial No. NCI-10-762(c)
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Metabolic Changes Associated with Cancer Surgery.

Principal Investigators: Dr. Alfred Ketchum, Dr. Robert Hoye, Dr. Howard Radwin, Dr. Edward McLaughlin, and Dr. Jack Bloch

Other Investigators: Surgery Staff, NCI

Cooperating Units: Dr. Milton Winitz of the Biochemistry Laboratory, NCI

Man Years (calendar year 1959):
Total: 1
Professional: 3/4
Other: 1/4

Patient Days (calendar year 1959):

Project Description:

Part 1. Blood volume alterations in patients undergoing radical cancer surgery. (Dr. Ketchum and Dr. Hoye)

Part 2. Effect of a synthetic water soluble diet on post-traumatic nitrogen balance. Effect of injury on growing animals. (Dr. Radwin)

Part 3. The effects of serum from patients with and without cancer upon hepatic regeneration in rats. (Dr. McLaughlin)

Part 4. Studies of the equilibrium rate of tumor water. (Dr. Bloch)

Objectives:

Part 1. In the surgical treatment of malignant disease many of our patients are admitted in a debilitated state. They are subjected to operative procedures lasting 6-8 hours and requiring 5-20 units of blood to cover operative loss. Their postoperative course is long and not infrequently complicated by avascular tissue sloughs, infection, oliguria, tachycardia, and many other metabolic alterations. The unreliability of the peripheral Hb., HCT., and RBC to reflect adequately changes in this type of patient's effective circulating blood volume has been

by Moore, Berlin, Williams, Parsons, and others. The need for a means of being able to assess the effective circulating red blood cell volume of these patients both in the preoperative preparation of the patient and in the postoperative phase is evident. Our immediate objective, therefore, is to develop a technique and facilities for the determination of the total red cell volume which would be both accurate and practical for use on our surgical ward.

Part 2. Major exenterative surgery, such as is performed by the Surgery Branch of the Cancer Institute, is disruptive to the general metabolism of the patient, and recovery is a burden to the reserves of those who are frequently already debilitated. The demands of convalescence might be eased if the patient were provided with the proper nutrients in the postoperative period. However, this would depend on the ability of the patient to utilize these nutrients. In this study a water soluble, synthetic, chemically defined diet developed at NIH is employed to discover in quantitative terms the value of a complete diet to the injured animal. Growth rate is followed as a measurable parameter of total body metabolism, and nitrogen balances are determined.

Part 3. Preliminary investigation has shown that the periodic intraperitoneal injection of serum from patients without cancer will inhibit liver regeneration of the white rat following partial hepatectomy. Serum from patients who have or who have had cancer will not produce this inhibition.

It is planned to use this basic data in studying patients who have cancer in the following ways:

1. To demonstrate if removing all or part of the cancer alters the patient's serum constituents as measured by hepatic regeneration.

2. To show whether or not a change occurs in the sera of cancer patients postoperatively and if such changes occur, what effects they have upon recurrence or metastases.

3. To demonstrate if such a procedure is of diagnostic value in the work-up of patients who have lesions in areas not accessible to excisional biopsy.

Part 4. To determine and compare the rates at which different animal and human tumors reach equilibrium with tritium labeled water in the plasma after intravenous administration of [illegible]

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Methods Employed:

Part 1. The technique presently being employed is the use of Cr^{51} as a red blood cell tag as recommended by Dr. H. Berlin. The red blood cells are tagged as whole blood and a weighed amount of this mixture injected into the patient. The standard sample is also prepared with a weighed amount of blood. Samples are drawn from the patient at 15 and 30 minute intervals. The amount of Cr^{51} in the blood and plasma of the standard and patient blood is determined on a scintillation counter. The total red cell volume is then calculated directly and the plasma volume indirectly using the peripheral HCT. as a factor. With this technique and by counting duplicate samples, our error is in the magnitude of 3-5%.

Part 2. Forty male Sprague-Dawley white rats were the experimental animals. They were subjected to varying dietary regimens both in quantity and in the method of feeding, some being force-fed and others permitted to ingest ad libitum. The experimental group was subjected to a crush injury to the femur producing a fracture on day 5 and were then further observed for nine postoperative days. Nitrogen balances and body weight were determined daily. At the conclusion of the experiment carcass analyses were performed on some animals.

Part 3. Venous blood is obtained. The serum is removed and stored at 40° F. until used. Female albino NIH rats, 180-270 grams, are subjected to a 70% partial hepatectomy. At eight hour intervals for 72 hours the animals are injected intra-peritoneally with serum. Seventy-two hours post partial hepatectomy, the animals are sacrificed and the degree of regeneration of the liver is calculated.

Part 4.

Animal Experiments: Tritiated water (HTO) in dosage of 100 mc. in 0.2 cc. is injected into the tail veins of 200-250 gram male Sprague-Dawley rats bearing a two to four week old Walker 256 carcinosarcoma subcutaneously on the back. Animals are sacrificed by intracardiac exsanguinating puncture at serial time intervals ranging from 5 minutes to 12 hours. Samples of blood, liver, muscle, tumor, and skin are lyophilized, and the water collected is measured for specific activity of tritium in the Packard Liquid Scintillation Counter. The ratio of tissue or tumor water/plasma water is recorded.

Human Studies: Tritiated water (HTO) in dosage of 200 mc. in 2 cc. is intravenously injected into human subjects with tumor, follow-

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drawing of a base-line blood sample. Following injection, serial blood samples are taken. Tumor is removed and time of excision during operative procedure recorded. Tumor and blood are lyophilized and the water extracted analyzed for tritium activity in the same manner as the previously described animal samples.

Major Findings:

Part 1. While improvements are still being made and new procedures being evaluated in our method of blood volume determination, we have noted the following significant findings:

1. Many of our patients on admission have a significant deficit in their total red cell volume, although this is not evident in their peripheral Hb. or HCT. determinations.

2. If an admission HCT. is relied upon as an indicator of effective circulating red cell volume prior to major surgery, as is not infrequently the practice, a patient may undergo major surgery with only 60% of his normal circulating red cell volume. Such a patient is a poor operative risk and has a difficult course.

3. By doing total red cell volume determinations immediately preoperatively and immediately postoperatively, we find there is a significant deficit of RBC's lost at surgery which has not been accounted for by the electronic measurement of blood lost in the operative field.

4. In the first two weeks following major surgery, there is a gradual continued loss of red blood cells. Twenty-five per cent of the total red cell volume may be lost in this way and not necessarily reflected in the patient's peripheral Hb. or HCT.

5. Patients developing a prolonged tachycardia in the immediate postoperative period with laboratory and clinical findings which are normal frequently have a 30-40% deficit in their effective circulating red cell volume and demonstrate a dramatic response following administration of two units of blood.

Part 2. The inhibition of growth by trauma was demonstrated in animals receiving a constant intake. A concomitant disturbance in anabolism was also observed. However, an increment of one third in the amount of diet available after injury could allay the inhibition of growth and minimize the alteration in nitrogen balance. While the utilization of diet is not as efficient in the post-operative period as in the preoperative, and the dietary supplement is not entirely retained, the provision of adequate nutrients is

worthwhile. Thus, while the effects of injury on the overall body economy as measured by growth rate are significant, they can be favorably modified by an appropriate dietary regimen.

Part 3. Selecting for study a series of consecutive admissions, it is hoped to demonstrate whether or not all patients with cancer show an inability to inhibit liver regeneration. Patients who have had no previous treatment, patients who have been treated but still retain evidence of their disease, and patients who have been treated and are thought to be free of their disease, will be studied. Preliminary work indicates that all three groups of patients exhibit an inability to exhibit liver regeneration; however, a larger series will be studied.

Obtaining serum from a large group of hospitalized patients one may determine if this defect in serum is specific for the patient with cancer or if it is present in other disease states. Should this prove to be of significant accuracy, such a serologic test would be of value in certain lesions which do not lend themselves to easy excisional biopsy; i.e., lung, stomach, pancreas, kidney, and cerebral tumors.

Preliminary data in fifteen patients in whom the diagnosis of carcinoma of lung, tuberculosis, anthrosilicosis, and aneurysm of thoracic aorta, were proven at thoracotomy, indicates that those patients with lung cancer showed a lack of inhibition of liver regeneration, while all non-cancer patients inhibited regeneration.

Obtaining a serum sample immediately prior to surgery, three weeks postoperatively, and at subsequent three month or six month intervals, one may demonstrate if there is a change in the individual patient's ability to inhibit liver regeneration. Such data could be correlated to the appearance of metastatic foci, recurrence of tumor, or death from cancer spread.

Preliminary results show:

1. Serum from patients with cancer does not inhibit liver regeneration.

2. Serum from patients without cancer does inhibit liver regeneration.

3. Serum from patients who have had cancer and who after surgery are believed to be free of their disease does not inhibit liver regeneration.

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4. In a series of 55 patients, all those with cancer did not inhibit liver regeneration. Those who did not have cancer (but had other diseases; i.e., tuberculosis, arthritis, diabetes, anthrosilicosis, wound infection, empyema, gout, etc.) inhibited liver regeneration.

Part 4. Animal experiments to date include 32 rats. 80-100 gram tumors in these animals take an average of 45 minutes to reach equilibrium with plasma tritium, 15 minutes longer than any other tissue studied. One metastatic cancer in the chest wall equilibrated in 90 minutes.

Significance to Cancer Research:

Understanding of fundamental problems of etiology and prognosis of cancer requires more precise information of metabolic changes associated with or produced by the disease. The studies outlined allow fundamental observations on cancer in man. This should help the surgeon prepare patients for surgery and perform surgery under the most optimum conditions. Dr. McLaughlin's interest in liver regeneration could possibly lead to a diagnostic test for cancer. It more likely will lead to more basic information of fundamental changes in serum protein (?) or other body constituents associated with cancer. Preliminary information indicates that patients with cancer lack a substance which inhibits liver (mitosis) regeneration. This substance is present in patients who do not have cancer. Conclusive proof of this preliminary information would be of significant value.

Proposed Course of Project:

The four projects outlined are all actively under study. It is believed that they will all be enlarged and completed. The exact course will depend upon individual developments.

Part B included Yes [ ] No [XX]

Serial No. NCI-10-765
1. Surgery Branch
2.
3. Bethesda, Md.

HHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of Carcinogenic Agents Using a Bronchial Pouch Preparation in the Dog.

Principal Investigator: Dr. W. C. Hueper

Other Investigators: Dr. William Benfield and Dr. Alfred Ketcham

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: 0

Patient Days (calendar year 1959):

Project Description:

Objectives:

1. To introduce and utilize the blind pouch technique into bronchogenic carcinoma carcinogenesis study. Results may be added to current evidence concerning the etiology of human lung cancer.

2. To provide a satisfactory large animal tumor for [illegible] studies.

Methods Employed:

As performed at present, the pouch is fashioned from the left lower lobar bronchus. A left lower lobectomy is first performed, amputating the bronchus more distal than usual. The lower lobe bronchus is then re-amputated at a higher level, severing the cartilaginous ring and endobronchial structures, but leaving the peribronchial structures intact as a vascular pedicle. The left main bronchus is sutured as are both ends of the pouch, forming in effect a pseudocyst. The end of a fine polyethylene catheter is implanted into the lumen of this pouch, and the other end of the catheter is exteriorized through the chest wall where it is buried beneath the skin, available for injection purposes.

Suspected carcinogenic agents will be injected into the bronchial pouch via the polyethylene catheter at regular intervals. Suitable controls where the vehicle only is injected will be included. Serial roentgenograms will be taken to detect and follow the progress of any left hilar radiodensity which may develop. The animals will be followed for four to five years, after which time they will be sacrificed. It is proposed to study the following agents initially: 3,4 benzpyrene, tobacco tar, and internal combustion engine exhaust concentrate. These three compounds are being injected into different animals, and a fourth group of control animals are being observed.

Major Findings:

This project was instituted and carried on by Dr. John Waite until his transfer to another Public Health Service Hospital. It is being completed by Dr. Alfred Ketchum, Surgery Branch and Dr. William Benfield of the Pathology Department.

The animals have been followed at three-month intervals with re-injection of tobacco tar into their bronchial pouches, and clinical evaluation of all animals within the experiment, which includes chest x-ray evaluation. This is intended to be carried out throughout the course of the study which extends over the next two years. The 1959 x-ray evaluation of the remaining dogs failed to show any significant findings. One animal has died within the past year and one has been sacrificed. The pathological specimens, both grossly and microscopically, have failed to show any evidence of cancer. There are presently 20 dogs remaining in the study.

To date there have been no significant results, either in the sacrificed animals or the clinical and radiographic findings in the living animals.

Significance to Cancer Research:

To date the majority of evidence purporting to bear on the genesis of human bronchogenic carcinoma is adduced from the ability of a suspected carcinogenic agent to produce a tumor when painted on the skin of a rabbit or mouse. In interpreting the results of these experiments, not only must the heterogeneicity of the experimental animal with respect to humans be considered, but dissimilarity between epidermis and bronchial epithelium plays an important role. Exposing the experimental animal to an atmosphere laden with the suspected carcinogenic agent has almost always been unsuccessful in producing tumors. This had led to the conclusion by some investigators that the agents are not respiratory carcinogens and by others that the animal's respiratory defenses (the cough, the dilution of the carcinogenic agent in the respiratory secretions, and the actions of cilia) are so efficient as to make exposure to the carcinogenic agent negligible using this technique. There is much logic to support all

around hypothesis, and a good method to provide contact with a strong concentration of the suspected agent over a prolonged period of time and against which contact the animal may not have an effective defensive mechanism might be of some value in settling this point. We feel that a study on the uptake of radioactive benzpyrene from our pouch preparation alone might yield sufficient data to justify the project.

Proposed Course of Project:

This project is being completed by Dr. Alfred Ketcham, Surgery Branch, with the assistance of Dr. Banfield. The animals are evaluated at three month intervals, but due to inability to obtain the tobacco tar from Dr. Hueper, re-injection of the one remaining patent polyethylene tube has not been carried out within the past six months. During the next two years these animals will be intermittently sacrificed as the protocol calls for and followed until the end of the experiment in 1961.

Part B included: Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Relationship of Uropepsin Excretion to Gastric Acidity and Pepsin.

Principal Investigator: Dr. James R. Jude

Other Investigators: Dr. Alvin Harris

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: 0

Patient Days (calendar year 1959):

Project Description:

Objectives:

To determine whether or not there is a constant and definite relation between uropepsin excretion and gastric pepsin and acid production in dogs. This value, if found to show a constant relationship to gastric acidity, would provide a valuable tool in clinical management of patients with gastric diseases.

Methods Employed:

Heidenhain and/or Pavlov pouch dogs have been prepared. The same animals have had cannulae placed in their urinary bladders. Simultaneous 24-hour collections from the urinary and gastric pouches shall be made over a control period of four weeks. The gastric juice volume, free and total acidity, and pepsin output shall be determined as also will the output of uropepsin. The data will be evaluated.

Following adequate control periods various experimental regimens are followed including:

1. Administration of cortisone acetate intramuscularly in daily doses of varying amounts over specified time periods.

2. Histamine in Beeswax administration.

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Serial No.

3. Hypophysectomy.

Major Findings:

The day to day 24 hour excretion of Heidenhain pouch free acid and pepsin and urinary bladder pepsin varied from 400% to 700% in maximum-minimum ranges. Histamine produced an immediate rise in gastric pouch free acid and pepsin, and a brief late increase in uropepsin. Large doses of cortisone produced variable increases in gastric free acid and a late gradual increase in gastric pepsin. No constant change in uropepsin was observed. After hypophysectomy a marked decrease in pouch acid and pepsin was seen. Again uropepsin excretion values did not vary from the pre-hypophysectomy control values.

It was concluded that the control studies gave strong indication of the lack of value of uropepsin excretion as a mirror of gastric mucosal activity in free acid or pepsin production. Neither increased stimulation with injection of histamine, and large doses of cortisone, or decrease in stimulation by ablation of pituitary produced the corollary effect on uropepsin. It can only be concluded that in the experimental animal used no value could be given to evaluation of gastric mucosal activity in acid and pepsin production by study of uropepsin excretion.

Significance to Cancer Research:

The literature contains many reports of the value or lack of value of uropepsin excretion in evaluating the secretory status of the stomach. If a simplified test could be found that would easily detect limited gastric acid secretion in patients, the test would be of great clinical value in detecting early gastric cancers, or patients who might be expected to develop gastric cancer. Unfortunately, this study reports only negative results.

Proposed Course of Project:

No further work planned.

Part B included: Yes [XX] No [ ]

Serial No.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Jude, James R. and Harris, Alvin H.: A Study of Dyspepsia as a Reflection of Gastric Secretory Activity. Arch. Surg. 79:5, 761-768, 1959.

Honors and Awards relating to this project: None

Serial No. NCI-10-773
1. Surgery Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calender Year 1959

Part A.

Project Title: Study of Host Factors Associated with Implantation of Cancer Cells.
Part I. Effect of Altered Endocrine State on Implantation and Growth of Tumor Emboli
Part II. Effect of Deficiency States on Cancer Immunity
Part III. Effect of Stress, Liver Trauma Upon Tumor Metastases
Part IV. Attempts to Delineate Host Factors Associated with Prognosis in Cancer Patients

Principal Investigator: Dr. Robert R. Smith

Other Investigators: Dr. Alfred Ketcham, Dr. Marvin Arons, Dr. Robert Couch, and Dr. Alan Retik

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2-3/4
Professional: 1-1/4
Other: 1-1/2

Patient Days (calendar year 1959):

Project Description:

Objectives:

Part I. (Dr. Arons and Dr. Ketcham) To determine the effect of pituitary hormones, growth hormone and ACTH, on primary and metastatic tumor growth. To observe the biology of a tumor previously hormonally treated and then transplanted.

Part II. Homotransplantation in Pyridoxine Deficient Mouse (Dr. Couch) The impaired immune response of the pyridoxine deficient animal has been well demonstrated. Utilizing this fact, Axelrod, Fisher, and Fisher were able to achieve significant success with homologous skin transplants in animals. The factors involved in the host immune response to cancer have been an interest of the surgical branch since its inception. In line with the work under way in patients on inhibition of host immunity to infection, it seemed reasonable to see if host immunity to cancer using a genetically pure system could be inhibited. Using this as a starting point, studies designed

ground discerning the factors involved in this immune response are planned. The ultimate hope is that discoveries will be made that will allow the creation of host immunity rather than the destruction of it. The results presented here are preliminary and incomplete.

Part III. (Dr. Retik) To determine the effect of stress and liver manipulation upon the metastases of S-91 melanoma and other mouse tumors.

Part IV. (Dr. Retik and Dr. Smith) To determine retrospectively and if possible prospectively factors, aside from the extent of a patient's disease, affecting the prognosis of patients with carcinoma of the cervix. Attempts to correlate changes in specific constituents of serum (i.e., serum glycoproteins) in patients that are able to contain their cancer and those that develop disseminated cancer will be included in this study.

Methods Employed:

Part I. (Dr. Arons) Though the pathogenesis of tumor metastases has been fairly well delineated histologically in recent years, yet the basic regulatory influences on the metastatic process are not yet well understood. All the hormones, especially the adrenal cortical group, have been under thorough investigation using various types of transplantable animal tumors. Despite voluminous experiments with the adrenal steroids, namely cortisone, with no less than 35 definitive references, many of the conclusions are contradictory regarding site of action, effect, and underlying cause. It has been noted in several good review articles that there are many pitfalls using animals in tumor experiments, especially when hormones are introduced; but with these factors in mind, the two pituitary hormones, growth hormone and ACTH, have been selected for investigation of their influence on experimental transplantable tumors. Of the hormones so far studied, probably the least work has been reported on growth hormone. It must be recognized that earlier growth hormone preparations have not been as pure or assayed as high as those most recently employed. Also, growth hormone must be administered [illegible] at intervals to counteract refractoriness, and in several experiments this was not done. Another interesting factor is that in contrast to the intact rat, the intact mouse is rather unresponsive to growth hormone except when hypophysectomized. However, this may be overcome by increasing the dose about 20 times. Summarizing these experiments, it has been concluded that growth hormone is without local effect on three different mouse adenocarcinomas, one mouse osteogenic sarcoma, and a rat fibroadenoma. However, one experiment concluded that the primary tumors themselves increased in size, and in this experiment two adenocarcinomas and two fibroadenomas

were used in mice. Regarding metastases, one paper concluded that there was no increase in lung metastases when growth hormone was administered to mice with an adenocarcinoma. Another experiment demonstrated increased lung metastases, but only when growth hormone was given intravenously and only when administered prior to tumor inoculation. However, he later showed that this was a nonspecific effect. One other point to be noted is that the average tumor mass attained in mice therapied with growth hormone in one paper is independent of body weight. Further, the growth hormone increased tumor weight and body weight. The tumor weight was characteristic of the specific tumor host combination. As for the effects of ACTH on various animal tumors the following conclusions have been made: 1) ACTH increases local tumor growth after hypophysectomy, 2) it decreases local tumor growth, 3) ACTH increases metastases when administered prior to intravenous tumor inoculation, 4) it has no local effect on tumor, 5) ACTH decreases local tumor growth and decreases metastases when used in conjunction with x-ray treatment, and 6) it increases the per cent of tumor take. It should be mentioned that the simultaneous administration of both growth hormone and ACTH has been shown to reverse the body weight loss produced by ACTH alone. With this confused back drop it was felt further experimentation might clarify the hormonal effects on local growth and metastases in transplantable animal tumors, and since only one investigator has reported on transplanting tumors from hormonally treated animals to observe if the hormone effect is continued in nontreated animals, this aspect of hormonal influence was also to be investigated. Therefore, an initial pilot study was undertaken, and two different experiments were carried out.

Experiment 1: Into four groups 60 six week old DBA gray mice were equally divided, and the animals were then weighed in groups of five. .03 cc. of a 1:10 dilution of a cytosieve DBA T-49 sarcoma was then given into the right hind limb intramuscularly. Immediately after tumor inoculation, all the animals received the following subcutaneous injections into the dorsal neck: Group 1 - .5 cc. distilled water, Group 2 - .5 mg. growth hormone, Group 3 - .05 mg. ACTH, Group 4 - .5 growth hormone and .05 mg. ACTH. The ACTH was Organon's corticotrophin zinc and the growth hormone was Armour's Somar A diluted with distilled water. Each animal in Group 2 and Group 4 received a total of 22 mg. of growth hormone, but these having been increased every six days so that one day prior to sacrifice each animal was injected with 2 mg. Each animal in Group 3 and Group 4 received a total of 1.29 mg. of ACTH, the dose being increased once on the 13th day so that one day prior to sacrifice each animal was receiving .08 mg. Each animal in Group 1 control received .5 mg. distilled water daily. Each group

of animals was housed together in four separate cages and all the animals were maintained on water and chow ad lib. Drug injection and tumor inoculation began on day 0, injections ending on day 20. Just prior to sacrifice on day 21, the animal weights were again recorded. The lungs were treated and stained by the method of Wexler and the number of metastases per lung counted using magnifying loops.

Experiment 2: Into four groups 40 six week old DBA grey mice were equally divided. A primary tumor from one animal of each of the four groups of Experiment 1 was cytosieved as previously described. These tumors were taken at the completion of this first experiment. Each tumor was then inoculated intramuscularly into the right hind limb of the animals in the corresponding group on day 0. None of the mice in any of the four groups received any drug and all were maintained as in Experiment 1. On day 22 all animals were sacrificed and the lungs examined for metastases by the previously described technique. The primary tumors were weighed individually.

Part II. (Dr. Couch)

Materials:

1. Animals - Balb/c 20 gram mice.
2. Diet - Water soluble diet of Greenstein, et al, for Experiments 1 and 2. Nutritional Biochemical Company B_6 deficient diet for Experiment 3.
3. Tumor - C_3HBA mammary tumor.
4. Desoxypyridoxine - Where used, given at 0.3 mg./cc. water.

Experiment 1: Ten control and ten experimental animals were started on diet. Control animals received chow diet and water ad lib. Experimental animals received diet #26 of Greenstein and water ad lib containing desoxypyridoxine. At the end of 3 weeks all animals were given a trocher injection of tumor into axillary and sacrificed 7 weeks after the study commenced. Tumors were submitted to pathology for sectioning.

Experiment 2: A repeat of Experiment 1 was done using 20 animals in each group. At 3 weeks diet was changed from [illegible].

Experiment 3: Three groups of animals were used. Twenty control animals were given chow diet and water ad lib. Twenty experimental animals were given NBC B_6 deficient diet and water ad lib. Twenty experimental animals were given pyridoxine deficient diet plus 0.3 mg. desoxypyridoxine per cc. of water. Animals were inoculated with tumor at 3 and 6 weeks. Sacrifice was done 4 weeks after inoculation.

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Part III. (Dr. Retik) Suspensions of S-91 melanoma were injected into the portal vein of the mice. The study is in progress now. After varying periods of time, repeat exploratory laparatomies and liver manipulation will be performed as well as other methods of stress to determine the effect on the incidence and number of liver metastases.

Part IV. (Dr. Retik and Dr. Smith) This project is most difficult to get organized. It was originally hoped that during this year studies would be under way to measure 3 fractions of human serum, glycoprotein, seromucoid, and C-reactive protein, in patients with operable malignant tumors. Dr. Jack Bloch did set up the following study and was able to standardize his techniques. 15 cc. of serum will be obtained from each patient to be studied and from each control subject, and will be stored under refrigeration until use. Levels of serum glycoprotein will be measured by the thymol sulfuric acid method of Shetler, in the Beckman D.U. Spectrophotometer using standard solutions of equimolar mannose and galactose as indices. Seromucoid will be measured with Folin's phenol reagent for tyrosine, in the Klett Colorimeter. C-reactive protein will be accurately estimated by the capillary precipitin method, using commercially prepared C-reactive protein antiserum.

In addition to this, Dr. Retik has initiated a chart review of patients who have had surgical treatment of carcinoma in an effort to delineate factors such as infection, multiple transfusions, stress, etc., that would be correlated with prognosis.

Major Findings:

Part I. (Dr. Arons) Since this was only a pilot study, no definite conclusions can be made, but only inferences drawn for the next experiment which will duplicate these two experiments.

Experiment 1: It is seen that the median weight of the primary tumors at sacrifice in each of the four groups weighed about essentially the same. The lesser weight in the ACTH treated animals probably represents the total effect of total body weight loss and also these tumors were somewhat liquified. It appears that growth hormone, ACTH, and the combination of the two hormones does not increase the growth of the primary tumor. The mean weight change in the Group 1 control animals about equals the weight of the tumor, whereas in the animals receiving growth hormone the local tumor does not account for the increase in body weight, which must be attributed to the growth hormone effect. When compared with the control value for median number of metastases per lung, ACTH in Group 3 and the ACTH-growth hormone combination in Group 4

compare favorably and do not seem to increase pulmonary metastases or decrease them. Yet in the growth hormone treated animals there appears that there might be a significant increase in lung metastases in these animals. In the Group 4 animals which receive both ACTH and growth hormone it is noted that the growth hormone counteracted the weight loss effect of ACTH seen in Group 3, but that the ACTH seemed to counteract the growth hormone effect of increasing pulmonary metastases.

Experiment 2: It is immediately noted that in Group 4 there was a 0% tumor take. This was the group in which the tumor was taken from an animal previously treated with both ACTH and growth hormone. As a result, of course, there were no lung metastases, either. Whether this was a definite hormonally transferred effect or whether this was due to infection or some other cause remains to be further investigated. In Groups 1, 2, and 3 the median weights of the primary tumor at sacrifice compare fairly equally. However, in comparing median numbers of metastases per lung it is noted that in the control the figure is 7, whereas in the growth hormone group it is 3, and in the ACTH group it is 0. Whether the tumors in Groups 2 and 3 that had been in animals previously hormonally treated were transferred with some hormonal effect on inhibiting metastases remains to be seen, especially since in the first experiment growth hormone seemed to increase metastases. In our next contemplated experiment we hope to duplicate these two experiments and also to use another control in Experiment 1, that being denatured growth hormone.

Part II. (Dr. Couch)

Experiment 1: No control animal exhibited "tumor take" either gross or microscopic. Two of seven surviving deficient animals exhibited gross and microscopic take.

Experiment 2: No control or experimental animal exhibited gross or microscopic tumor. Tumor inoculation time coincided with diet change and marked weight loss occurred in both control and experimental groups.

Experiment 3: No control animal exhibited tumor. All [illegible] animals receiving deoxypyridoxine did not survive longer than 7 weeks post injection. B_6 deficient animals without antagonist survived. One of seven animals in the 3 week group and three of seven animals in the 6 week group exhibited "tumor take".

Parts III & IV. No significant results available. [illegible] is in progress.

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Significance to Cancer Research:

The clinical experience with human cancer therapy indicates that control of cancer, once it develops, depends upon the ability to control the implantation of cancer cells both locally in a wound and at distant sites. There is increasing evidence that more than the mere presence of tumor cells at a site of potential recurrence or metastases is necessary for the growth of these cells. If it could be shown that altering the endocrine level of patients could influence this host factor, or if a measurable serum factor could be correlated with metastasibility, a potent cancer control method would be available.

The details of the chemical and physiological nature of the serum polysaccharides are largely unknown, but it has been demonstrated by numerous investigators, that in many disease states the measurable serum levels are altered. Over the past twenty years, Shetler et al have studied the serum levels and properties of the "acute phase reactants", notably serum hexose or glycoprotein, its close relative seromucoid, and C-reactive proteins, in cancer, many of the collagen diseases, pregnancy, non-specific fever, and experimental inflammation, and have noted significant rise of these fractions in the above states.

The relationship of the rise in these serum polysaccharides to the disease process has not been postulated. It is known that the levels are significantly elevated in active cancer, that they rise still higher immediately postoperatively, and in a few followed cases have declined postoperatively. We are interested in attempting to correlate the biological behavior of carcinoma in our attempt at operative cure of this disease, with serum polysaccharide levels.

Proposed Course of Project:

It is difficult to delineate the exact course this project will take. All of the above described protocols are incomplete and no significant results are available. The course of this study is dependent upon findings of pilot experiments still in progress.

Part B included: Yes [ ] No [XX]

To be issued at a later date.

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